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Role of Nest Raiding and Egg Predation in Regulating Population Density of Threespine Sticklebacks (*Gasterosteus aculeatus*) in a Coastal British Columbia Lake

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Hyatt, K. D., and N. H. Ringler. 1989. Role of nest raiding and egg predation in regulating population density of threespine sticklebacks (*Gasterosteus aculeatus*) in a coastal British Columbia lake. Can. J. Fish. Aquat. Sci. 46: 372–383.

The consequences of nest raiding and egg predation for population regulation of sticklebacks (*Gasterosteus aculeatus*) were studied in a British Columbia coastal lake. Stickleback eggs were present in the stomachs of 23 and 11% of all sticklebacks sampled from the field in 1982 and 1985 respectively. On average, males consumed more eggs than did females. Seasonal egg consumption increased with increases in stickleback density in breeding colonies. In experimental enclosure studies, nest densities varied between 0 and $3 \cdot m^{-2}$ as stickleback numbers were manipulated from 1.5 to $12 \cdot m^{-2}$. Peak nest densities occurred at 5.8 fish$\cdot m^{-2}$. Stickleback density explained 70–86% of the variation in nest numbers in an application of a Ricker stock–recruit model and weekly rates of nest survival exhibited strong density dependence. Our evidence indicates that both nest raiding and egg cannibalism are important in limiting the production of stickleback fry as population density increases in scattered, lakeshore, breeding colonies of Kennedy Lake.

Les conséquences de la déprédation des nids et de la prédation des oeufs sur la régulation démographique de l'épinoche à trois épines (*Gasterosteus aculeatus*) ont été étudiées dans un lac côtier de la Colombie-Britannique. Les oeufs d'épinoche ont été trouvés dans l'estomac de 11 et 25 % de toutes les épinoches échantillonnées en 1982 et 1985, respectivement. En moyenne, les mâles avalent davantage d'oeufs que les femelles. La consommation saisonnière des oeufs augmente avec la densité d'épinoches dans les colonies réunies pour la reproduction. Dans des enceintes, on a fait varier la densité des nids entre 0 et $3 \cdot m^{-2}$ en faisant varier la densité des épinoches entre 1,5 et $12 \cdot m^{-2}$. La densité maximum de nids a été observée à 5,8 poissons$\cdot m^{-2}$. La densité des épinoches explique à 70–86 % la variation du nombre de nids dans un modèle de recrutement des stocks de Ricker; le taux hebdomadaire de survie des nids dépendait fortement de la densité. Nos résultats indiquent que la déprédation des nids et le cannibalisme réduisent de façon importante la production d'alevins d'épinoches à mesure que la densité de population augmente dans les colonies qui se forment près des rives du lac Kennedy pour se reproduire.

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Threespine sticklebacks (*Gasterosteus aculeatus*; various phenotypes including unplated, partially plated, and fully plated) are sometimes a dominant component of the fish communities of coastal lakes in British Columbia. Recent field enclosure experiments (O'Neill and Hyatt 1987) have shown that sticklebacks can rapidly modify the standing crop, size, and species composition of zooplankton in coastal lakes. In about half of such lakes examined by Hyatt and Stockner (1985) "limnetic" sticklebacks were potential competitors with juvenile sockeye salmon (*Oncorhynchus nerka*). Further, the density of some stickleback populations increased following lake treatment with ammonium nitrate and ammonium phosphate, which was added to enhance growth and survival of juvenile sockeye salmon (Hyatt and Stockner 1985).

Remarkably little is known of the population dynamics of threespine sticklebacks, despite interest in most other aspects of their biology for more than a century (e.g. Wootton 1976, 1984). We were interested in mechanisms that act to limit population density in the extremely oligotrophic lakes of coastal British Columbia (Stockner and Shortreed 1985). Since 1977, the densities of sticklebacks and juvenile sockeye salmon in the limnetic zone of several coastal lakes have been estimated by hydroacoustic and midwater trawl techniques (Hyatt and Stockner 1985). Seasonal changes in the abundance of sticklebacks in the littoral zone of these same lakes have been indexed by using catch rates in standard-sets of minnow traps (Hyatt et al. 1984). In about half of the 16 lakes surveyed, sticklebacks remained in the limnetic zone and fed only on zooplankton during fall and winter.

In Kennedy Lake, B.C. an extensive onshore migration to littoral nesting habitats begins in late April or early May, and peaks from mid-July to early August. Nesting usually continues until late August or September after which all sticklebacks move to the limnetic zone.

Analysis of stomachs from littoral-dwelling sticklebacks, trapped in Kennedy Lake during 1982, indicated that eggs of

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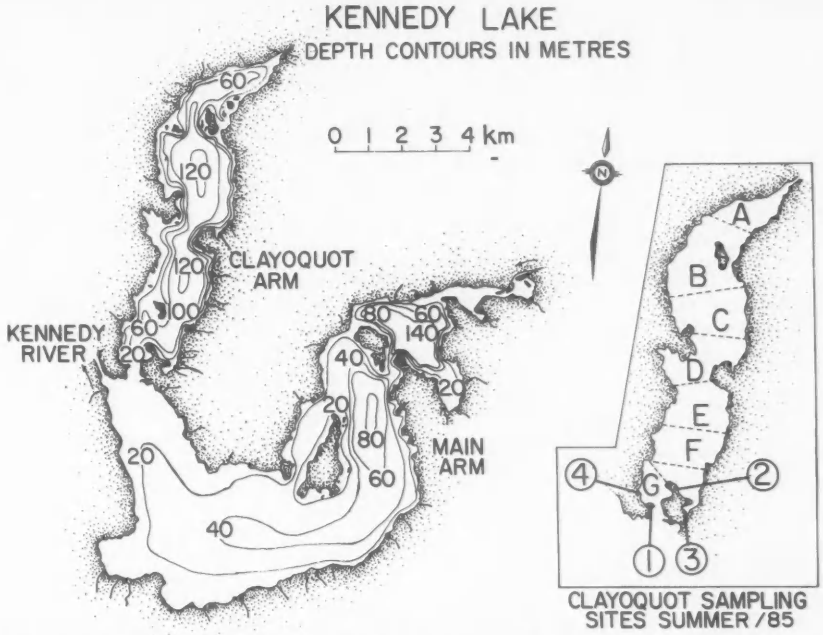

FIG. 1. Bathymetric map indicating hydroacoustic and trawl transects (A–G), trapping sites (1–3), and the site of experimental enclosures (four) used for studies of sticklebacks in 1982 and 1985 at Kennedy Lake, Vancouver Island, B.C.

conspecifics were important in the diet. Nest raiding and egg consumption by sticklebacks have been described and interpreted both in ethological and ecological contexts by Hynes (1950); Van den Assem (1967); Wootton (1971); Rohwer (1978); Li and Owings (1978); Kynard (1978); Ridley and Rechten (1981); and others. Whoriskey and FitzGerald (1985a) reported that when territorial males were experimentally removed from their nests in tidepool habitats, only 11 of 58 nests (19%) were still intact after 24 h. Our observations in Kennedy Lake over several years indicated that raids on defended nests by individuals and especially groups of sticklebacks were a common occurrence during the breeding season.

We hypothesized that reproductive success of *G. aculeatus* might be greatly influenced both by changes in the onshore abundance of sticklebacks, as successive waves of fish recruited there from the limnetic zone, and by seasonal changes in the tendency of sticklebacks to eat the eggs of conspecifics. If nest raiding and egg predation were density-dependent phenomena as shown by Whoriskey and FitzGerald (1985b), they could be important mechanisms for regulation of stickleback population size in coastal lakes. We tested this hypothesis by (1) monitoring seasonal change in adult population size and diets of sticklebacks in Kennedy Lake, and by (2) experimental manipulation of the density of reproductively active sticklebacks in enclosures maintained in the littoral zone of the lake.

Study Sites

Our work was carried out in Clayoquot Arm of Kennedy Lake (Fig. 1), situated on the West Coast of Vancouver Island,

TABLE 1. Characteristics of study sites on Clayoquot Arm.

Site number	Shoreline slope	Substrate texture	Vegetation cover	Substrate composition
site 1	20–40°	firm	*Scirpus* sp. *Equisetum* sp. *Lobelia* sp.	Mixture of detritus, sand and cobble overlying a matrix of rooted vegetation. Abundant woody debris.
site 2	20–30°	soft to firm	*Scirpus* sp. *Nuphar* sp. *Potomageton* spp.	Mixture of detritus and mud with some fine sand. Very little woody debris.
site 3	10–20°	soft	*Nuphar* sp. *Photomageton* spp.	A layer of fine detritus overlying mud. Abundant woody debris.
site 4	10–14°	firm	*Scirpus* sp. *Lobelia* sp.	Cobble and sand overlying a matrix of rooted vegetation. Moderate amounts of woody debris.

B.C. Clayoquot Arm is 1680 ha in surface area, with a mean depth of 49 m (Rutherford et al. 1986). It is connected by a narrow, 100-m channel to the main arm of Kennedy Lake, with which little exchange of nutrients or fish populations (except sockeye salmon) appears to occur. In order of numerical abundance the fish species in the lake are threespine sticklebacks, juvenile sockeye salmon, prickly sculpin (*Cottus asper*), peamouth chub, (*Mylocheilus caurinus*), cutthroat trout (*Salmo clarki clarki*), and juvenile coho salmon (*Oncorhynchus kisutch*). Stockner and Shortreed (1985) have established that Kennedy–Clayoquot, like the majority of B.C. coastal lakes, is characterized by short water residence time (<2 yr), low phosphorus concentration (3.0–6.0 μg TP·L^{-1}), and extremely oligotrophic conditions (3.1–11.2 μg TChl·L^{-1}). Littoral zone habitat (<5 m depth) is restricted to 3.2% of the surface area of the lake. The lake has been treated with ammonium nitrate and ammonium phosphate since 1979 as part of the Lake Enrichment Program (Hyatt and Stockner 1985).

Of more than 20 sites initially surveyed, we selected four littoral-zone sites for study in Clayoquot Arm (Fig. 1). The sites were qualitatively different with regard to substrate type and aquatic vegetation (Table 1), reflecting the range of littoral conditions suitable for nesting colonies of sticklebacks.

Methods

Population Density

The relative abundance of sticklebacks at site 2 was determined weekly from May 19 to August 3, 1982 and at sites 1–3 from April 4 to September 17, 1985. Groups of five Gee-type minnow traps were set 3–5 m apart for 5 h at depths of 0.25–0.50 m in 1982 and for 24 h at depths of 0.25–0.50 m and 1.0–1.5 m in 1985. Analysis of trapping records (Hyatt et al. 1984) has shown that maximum catches of sticklebacks in the littoral zone of these coastal lakes normally occur at depths ≤1.5 m. The density and total population size of nonbreeding sticklebacks during their limnetic phase were estimated during the interval from September of 1984 to January of 1985, through hydroacoustics and trawling techniques documented in considerable detail elsewhere (Hyatt et al. 1984; Hyatt and Stockner 1985; Gjernes et al. 1986).

Diet Analysis

All sticklebacks caught in the minnow traps were killed by momentary immersion in 95% ethyl alcohol, and preserved in 10% formalin or (in a few cases) 70% ethyl alcohol. For each fish we measured the total length, determined the sex, and removed the gut. The contents of the stomach were removed and stickleback eggs, if present, were counted at a magnification of 12 – 50× by using a Wild Stereomicroscope. In 1982, the dry weights of eggs and other prey items were determined on a Cahn electrobalance after samples from individual stomachs had been sorted, placed on preweighed, aluminum foil holders and subjected to 90°C for 24 h in a drying oven. In 1985 we estimated the volume of eggs and other prey items by placing them on a 1-mm deep, squash plate that had a series of grid volumes equivalent to 1 μL.

Experimental Manipulations and Nest Survivorship

Four enclosures were built along 30 m of the southwest shoreline of Clayoquot Arm (site 4: Fig. 1). This location was selected for the following reasons: trapping results and visual observations revealed a substantial population of nesting sticklebacks; the site was relatively free of the effects of brisk winds, which affect the accuracy of nest site identification; human intervention in the form of boaters and swimmers was absent; the substrate was firm enough to walk on with little disturbance, yet sufficiently free of boulders and woody debris to permit construction of effective enclosures; and the slope was sufficiently moderate to enable accurate observation of fish in all regions of the enclosures.

Each 3 × 3 m enclosure was constructed of 6–7.5-mm wire mesh, supported by 5-mm diameter steel rods. Construction was completed by 10 July, but the lakeward wall was left open until 12 July 1985. This procedure enabled us to confirm the existence of active nests at the time closure occurred. Water depth within the enclosures ranged from 0 to 40 cm; the walls were 60–80 cm high, as appropriate to ensure that fish could not escape or enter in association with moderate changes in lake level.

The enclosures were surveyed visually at least weekly from July 11 to August 20, 1985. A survey entailed 1.5–3 h of observation per enclosure over a 2-d period, during which nests were identified and mapped within 1-m grid squares. Courtship and aggressive behaviors were used to help locate territorial males. The major criterion for nest recognition, however, was the confirmation of nesting activities, including fan, push, bore, or dig, carry to, and hold station (Wootton 1972). A total of 35 behaviors associated with nesting and courtship were quantified in another phase of the study (Ringler and Hyatt, unpubl. data); we are confident that our nest surveys accurately represent nesting patterns within the enclosures. Active nests were marked with flags fixed to 2-mm diameter steel rods inserted approximately 10 cm from the nest opening. Flags were removed from inactive nests at the end of each survey.

Estimates of the total number of fish (nesters and nonnesters) were based on visual counts repeated by a single observer at least three times in each enclosure. Population densities in each enclosure were calculated from visual counts made 5–7 d before (PNOM) and 1 d after (NHAT) each survey, divided by the appropriate estimates of surface area and water volume.

Densities in the enclosures were manipulated by adding or removing sticklebacks at 2-wk intervals. Fish to be introduced were trapped overnight in Gee traps set in Kennedy Lake within 5 m of the enclosures, and placed in 1-m diameter holding pens according to sex. The fish were transferred by dip net from the pens to a bucket, and released immediately as a group along the shoreward wall of the enclosure. On all occasions except July 12, 1985 the sex ratio of the fish added was 1:1. Sex ratios on July 12 are unknown, but trapping records from July 10–29 suggest an estimate of 1.07 males : 1.0 females. Fish were removed on two occasions from enclosure D, with four traps fished for 4–5 h. Only males with nuptial colors were returned unharmed to the enclosure. The schedule of experimental manipulations and estimated numbers of sticklebacks per enclosure are shown in Table 2.

Analyses of nest density and survivorship were based on the following definitions: New nests, active nests identified in the latest survey but not during earlier surveys, regardless of whether the nests persisted through subsequent observations in the current week; old nests, active nests that survived from a previous week, regardless of whether they persisted through subsequent observations in the current week; total nests, new nests and old nests; lost nests, nests identified in a previous

week that were no longer being maintained or defended, based on at least two periods of observation within a survey. Cases in which nests were vacated during a 2-d survey were not scored as lost until the following week; survival rates, old nests/total nests in the previous week; extinction rate, lost nests/total nests in the previous week.

The statistical analysis system software (SAS Inc. 1982) was used to perform all data manipulations and calculations.

Results

Patterns of Abundance in Limnetic and Littoral Habitats

Hydroacoustic and midwater trawl surveys were conducted on seven transects in the limnetic zone of Clayoquot Arm of Kennedy Lake (Fig. 1, inset) on each of three occasions prior to the interval of active breeding during the summer of 1985. Results from these surveys clearly indicated the presence of two size and age groups of sticklebacks in the limnetic zone of the lake (Fig. 2). Serial sampling from early fall of 1984 through April of 1985, along with age determinations, based upon examination of otoliths (Hyatt, unpubl. data), indicated that the smallest size–group of sticklebacks (<50 mm in length) were juveniles produced during the 1984 breeding season. The larger size–group (>50 mm in length) consisted of at least two age–classes of adult sticklebacks that had bred on at least one prior

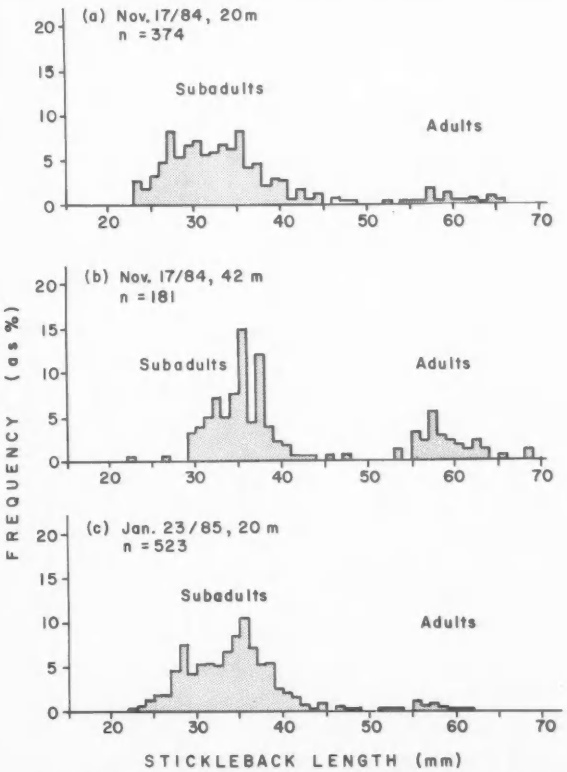

FIG. 2. Size–frequency distributions of sticklebacks captured in a mid-water trawl in the limnetic zone of Kennedy Clayoquot during the winter prior to the 1985 breeding season. Samples were obtained at one or more depths which the echosounder indicated harboured at least 85% of the overwintering population of fish.

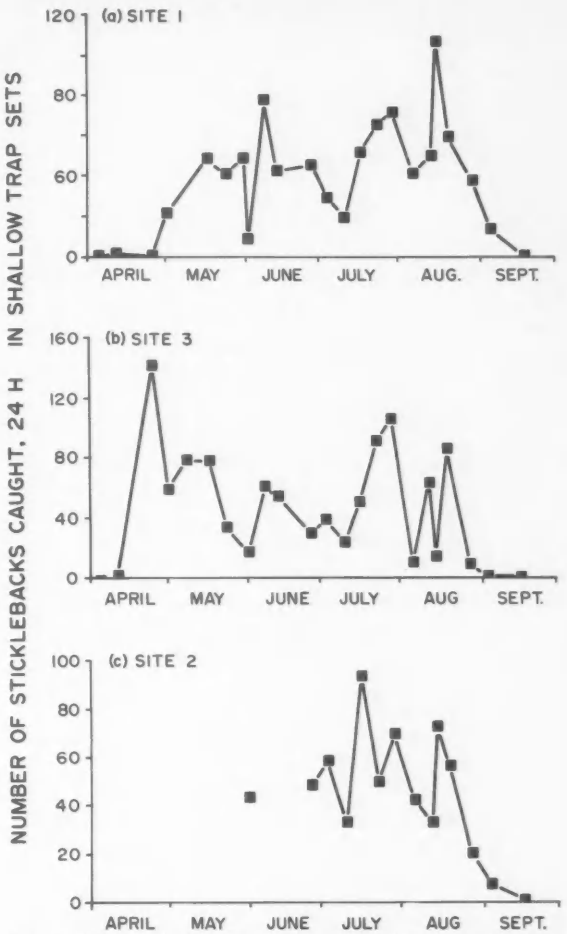

FIG. 3. Variations in the relative abundance of *G. aculeatus* in three shallow (0.25–0.50 m), littoral zone locations in Kennedy Clayoquot during the 1985 breeding season.

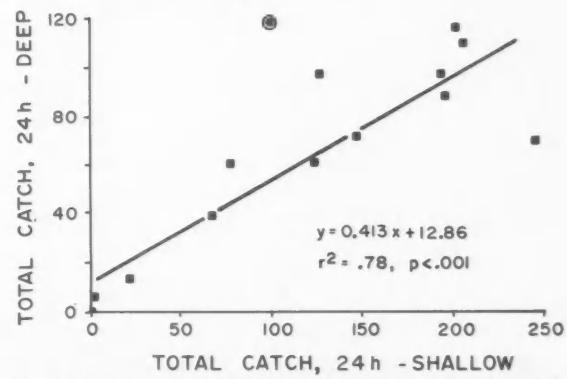

FIG. 4. Relationship between 24-h catches of *G. aculeatus* from shallow (0.25–0.50 m) and deep (1.0–1.5 m) trap sets. Catches are totals pooled across sites 1–3, at which matched shallow and deep sets were completed on 14 occasions from late June to October 1985. The circled observation was excluded from analysis due to storm driven turbulence on this date, which evidently forced temporary movement by many sticklebacks into deeper water.

TABLE 2. Schedule of experimental manipulations and estimated numbers of sticklebacks in enclosures, Clayoquot Arm, 1985.

	July 8–10	July 12	July 15–16	July 22–23	July 29–30	Aug. 5–6	Aug. 12–13	Aug. 19–20
Enclosure A								
Initial population	0	15	29	30	56	56	50	41
Manipulation	build enclosure	closure	none	add fish	none	none	none	none
Final population	0	15	29	68	56	56	50	41
Enclosure B								
Initial population	0	9	17	18	48	46	74	69
Manipulation	build enclosure	closure, add fish	none	add fish	none	add fish	none	none
Final population	0	27	17	54	48	128	74	69
Enclosure C								
Initial population	0	14	43	32	24	25	91	88
Manipulation	build enclosure	closure, add fish	none	none	none	add fish	none	none
Final population	0	50	43	32	24	103	91	88
Enclosure D								
Initial population	0	13	43	39	18	20	14	11
Manipulation	build enclosure	closure, add fish	none	remove fish	none	remove fish	none	none
Final population	0	67	53	17	18	14	14	11

TABLE 3. Hydroacoustic and trawl based population estimates of *G. aculeatus* in the offshore waters of Kennedy Clayoquot prior to the 1985 breeding season. Hyatt et al. (1984) and Hyatt and Stockner (1985) may be consulted for details of survey procedures and analytical methods, respectively.

			Hydroacoustic estimates			
Census date	Total No. of 20-min trawls	Total catch of sticklebacks	Stickleback population	95% CI	Adult	Subadults
Sept. 26/84	21	1407	5 941 691	1 266 122	1 112 339	4 829 352
Nov. 17/84	23	600	3 920 244	809 793	921 439	2 999 405
Jan. 23/85	21	564	2 941 633	724 387	512 894	2 428 739

TABLE 4. Summary of the contribution of stickleback eggs to the diet of Kennedy Lake sticklebacks during the 1982 and 1985 breeding seasons.

	1982			1985		
	Males	Females	Total	Males	Females	Total
Number examined	239	192	431	816	466	1282
% containing eggs	32.20	11.50	23.00	13.84	6.65	11.23
Mean (range) of eggs·fish^{-1} in egg-eaters	11.06 (1–54)	8.29 (3–20)	10.48 (1–54)	7.38 (1–30)	6.48 (1–29)	7.19 (1–30)
Mean dry weight (mg in 1982) or volume (μl in 1985)·fish^{-1} of all dietary items	2.29	2.39	2.34	7.85	5.36	6.95
Number of fish examined for diet dry weight or volume	92	107	199	780	445	1225
G. aculeatus eggs as % dry wt. or volume of *G. aculeatus* diet	39.83	17.05	27.40	38.87	20.05	33.59
Mean dry wt. (1982) or volume (1985)·fish^{-1} of all dietary items in egg-eaters only	5.50	6.95	5.97	30.36	24.09	29.01
G. aculeatus eggs as % dry weight (1982) or volume (1985) of diet of egg-eating *G. aculeatus*	72.73	62.63	68.90	69.40	64.12	68.45
Eyed eggs as % of all eggs eaten	4.10	1.14	3.59	0.03	0.06	0.03

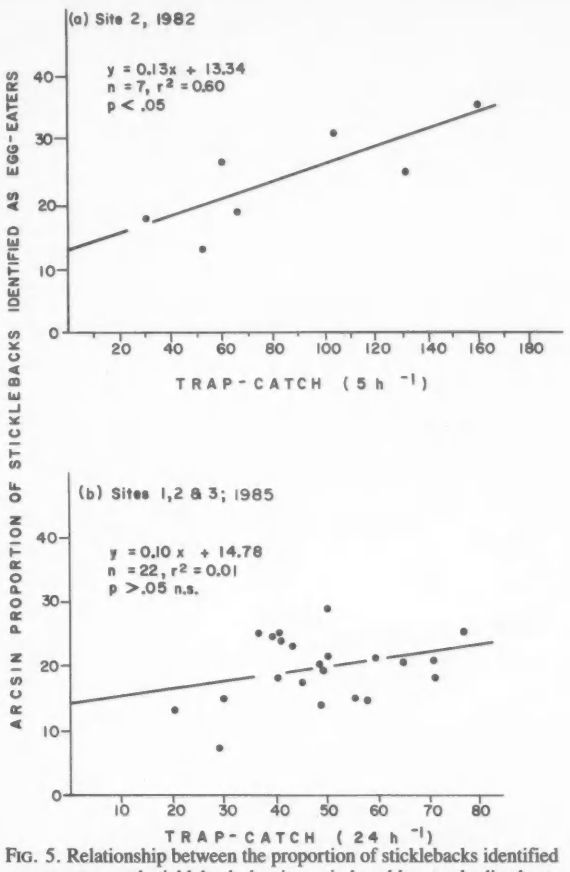

FIG. 5. Relationship between the proportion of sticklebacks identified as egg-eaters and stickleback density as indexed by standardized sets of five traps in breeding colonies at Kennedy Lake in (a) 1982 (samples from site 2 only), and (b) 1985 (samples pooled from sites 1, 2 and 3). Note that trap sets were of 5 h duration in 1982 and 24 h duration in 1985.

TABLE 5. Proportion of sticklebacks from 1985 classified as egg-eaters from samples obtained during intervals when 24-h trap catches of littoral-zone breeders indicated population levels were low (0–29 fish·set^{-1}), medium (30–59 fish·set^{-1}), or high (60–86 fish·set^{-1}). ($\chi^2 = 7.16$, $df = 2$, $p < .01$).

	Onshore abundance		
Egg-Eaters	low	medium	high
No.	13	99	38
Percent	6.40	12.12	14.07
N	203	817	270

occasion. Past observations have indicated that all fish from both size–groups would become sexually mature and spawn in 1985. Accordingly, the population of potentially reproductively active sticklebacks available immediately prior to the 1985 season in Clayoquot Arm consisted of 2.43 million novice breeders and 0.51 million repeat breeders (Table 3).

Breeding sticklebacks first appeared at shallow onshore sites in Clayoquot Arm in 1985 from mid to late April, after which several "pulses" of animals recruited onshore at irregular

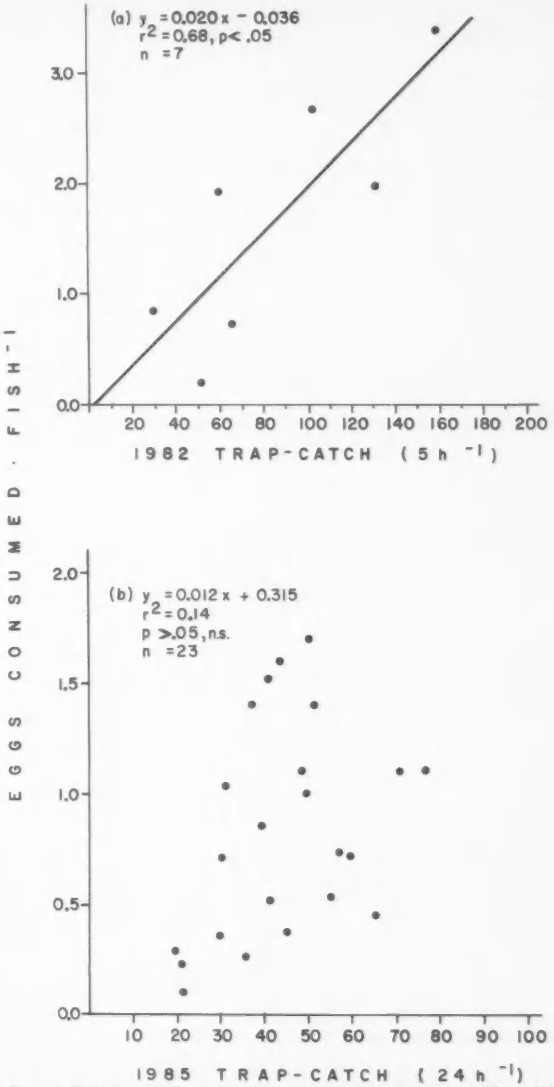

FIG. 6. Relationship between the number of eggs consumed by adult sticklebacks and stickleback density as indexed by standardized trap sets in breeding colonies at Kennedy Lake in (1) 1982, and (b) 1985. Dates, sampling sites, and effort levels as in Fig. 5.

intervals until mid-August (Fig. 3). Thereafter, the abundance of sticklebacks declined continuously such that by September 17 only two individuals were caught in 30 traps. This pattern of recruitment onshore to breed is similar to that observed in both basins of Kennedy Lake in a number of other years, although the breeding interval lasted 2–3 wk longer in 1985 than in previous years (K. Hyatt, unpubl. data), presumably due to an unusually long and warm summer. During 1985, the major breeding effort of the stickleback population appeared to last about 129 d.

Variations among the shallow site trap catches in Kennedy Clayoquot during 1985 suggest some between-site differences, but on the whole, patterns of onshore recruitment were similar. The most noticeable difference in onshore movement involved

TABLE 6. Dimensions of enclosures, population densities, and nest counts in enclosures in Clayoquot Arm, 1985.

		Dimensions		Population densities				Nest counts		
Enclosure	Dates	Area (m^2)	Vol. (m^3)	PNOM[a]/area	PNOM/vol.	NHAT[b]/area	NHAT/vol.	Total	"Old"	"New"
A	July 11–12	9.59	2.67	1.56	5.61	1.56	5.61	8	—	—
A	July 15–16	9.59	2.51	1.56	5.97	3.02	11.54	15	7	8
A	July 22–23	9.59	1.94	3.02	14.89	3.12	15.40	22	11	11
A	July 29–30	9.50	1.34	7.15	50.60	5.89	41.67	27	9	18
A	Aug. 5–6	8.18	0.78	6.84	71.07	6.84	71.07	21	15	6
A	Aug. 12–13	7.69	0.74	7.28	74.77	6.50	66.76	12	7	5
A	Aug. 19–20	6.08	0.39	8.22	125.60	6.74	103.00	6	4	2
B	July 11–12	9.69	2.63	0.92	3.41	2.78	10.25	10	—	—
B	July 15–16	9.69	2.46	2.78	10.95	1.75	6.89	13	10	3
B	July 22–23	9.69	1.88	1.75	9.04	1.85	9.57	12	9	3
B	July 29–30	9.69	1.35	5.57	39.76	4.95	35.35	23	8	15
B	Aug. 5–6	8.74	0.86	5.49	55.68	5.26	53.36	20	15	5
B	Aug. 12–13	8.00	0.69	16.00	183.90	9.25	106.30	17	5	12
B	Aug. 19–20	5.87	0.42	12.60	172.50	11.75	160.80	10	4	6
C	July 11–12	9.14	2.64	5.47	18.94	4.70	16.29	11	2	9
C	July 22–23	9.14	2.18	4.70	19.68	3.50	14.64	12	7	5
C	July 29–30	9.14	1.67	3.50	19.13	2.62	14.34	15	11	1
C	Aug. 5–6	8.93	1.15	2.68	20.87	2.78	21.74	13	10	3
C	Aug. 12–13	8.43	1.01	12.22	101.10	10.80	89.30	15	6	9
C	Aug. 19–20	7.14	0.64	12.74	141.30	12.32	136.60	15	4	11
D	July 15–16	9.36	2.21	7.16	30.22	5.66	23.91	8	2	6
D	July 22–23	9.36	1.79	5.66	29.53	4.16	21.73	12	6	6
D	July 29–30	9.36	1.47	1.81	11.52	1.92	12.20	10	6	4

[a]Number of sticklebacks estimated from repeated counts 5–7 d before nest survey.
[b]Number of sticklebacks estimated from repeated counts 1 d after nest survey.

site 3, a small, well protected embayment, which experienced recruitment of large numbers of mature sticklebacks at least a week in advance of the more exposed site 1 (Fig. 3a, b). Regardless, similarities among sites outweighed the differences. For example, pronounced peaks in fish abundance occurred at all sites on June 8 and July 28; and declines on June 1, July 9, and August 5 (Fig. 3a, b, and c). Further, strong correlation (simple linear correlation, $r^2 = 0.78$, $p < 0.001$) between catches obtained from shallow (0.25–0.50 m) and deep (1.0–1.5 m) trap sets, pooled across our three sites (Fig. 4), indicates that the onshore recruitment patterns apply to the entire littoral zone, not just the extreme shallows, in which the longest time series trapping was completed (Fig. 3).

These results indicate substantial synchronization of breeding activities at widely scattered sites so that large changes in the onshore density of breeding animals occur intermittently throughout the breeding season. Similar patterns of synchronization of breeding activities have been observed in other populations of sticklebacks (Van Mullem 1967).

Diet Analysis

A total of 431 fish (239 males, 192 females) were examined for egg-eating from a single site (site 2), throughout the breeding season in 1982. In 1985, 1282 fish (816 males, 466 females) were examined from three Clayoquot Arm locations (Fig. 1, sites 1, 2, and 3). The frequency of egg eating varied between years from 23.0% of the fish examined in 1982 to 11.2% in 1985 (Table 4). Twice as many sticklebacks consumed eggs during the 1982 breeding season as in the 1985 breeding season and more eggs were obtained by individual fish in 1982 (10.48 eggs·fish^{-1}) than during 1985 (7.19 eggs·fish^{-1}). In both years, 2–3 × more males than females included eggs in their diets and males on average consumed more eggs·fish^{-1} than females did (Table 4).

Due to their relatively large size, eggs constituted an important component of the overall diet of Kennedy Lake sticklebacks (27.4% by weight of the diet in 1982, 32.8% by volume in 1985) and were by far the largest component of the diets of egg-eaters (68.9% by weight in 1982, 63.9% by volume in 1985). Further, there appears to be an energetic advantage to egg eating as egg-eaters contained 2.4–4.5 × more food (by dry weight or volume) in their diets on average than did nonegg-eaters (Table 4).

In both sexes most eggs consumed (96.4% in 1982, 95.4% in 1985) were in relatively early stages of development (i.e. uneyed). By dividing either the total weight or volume of eggs by the numbers eaten, we obtained a measure of the size of the eggs consumed over the season. There was no evidence that the size of eggs consumed was related to the date of sampling and no clear relation between the number of eggs eaten and the date of sampling.

We examined the relationship between the proportion of sticklebacks that had eaten eggs and catch-per-unit effort in traps to test the hypothesis that egg-eating is a density-dependent phenomenon. Only observations from samples containing 20 or more sticklebacks were considered, to reduce random error associated with small samples sizes when identifying proportions of sticklebacks engaging in egg-eating. Our results suggest that the pattern of egg consumption is density related. In both years a significant increase in the proportion of egg-eaters occurred in association with increases in the onshore abundance of breeding sticklebacks (Fig. 5). The strength of this association varied markedly between years as continuous changes in onshore abundance accounted for a significant amount of the variation in the proportion of sticklebacks consuming eggs in 1982 ($r^2 = 0.60$, $p < 0.05$, Fig. 5a) but an insignificant amount of the variation in 1985 ($r^2 = 0.006$, $p > 0.05$, Fig. 5b). A Chi-square test applied to the 1985 obser-

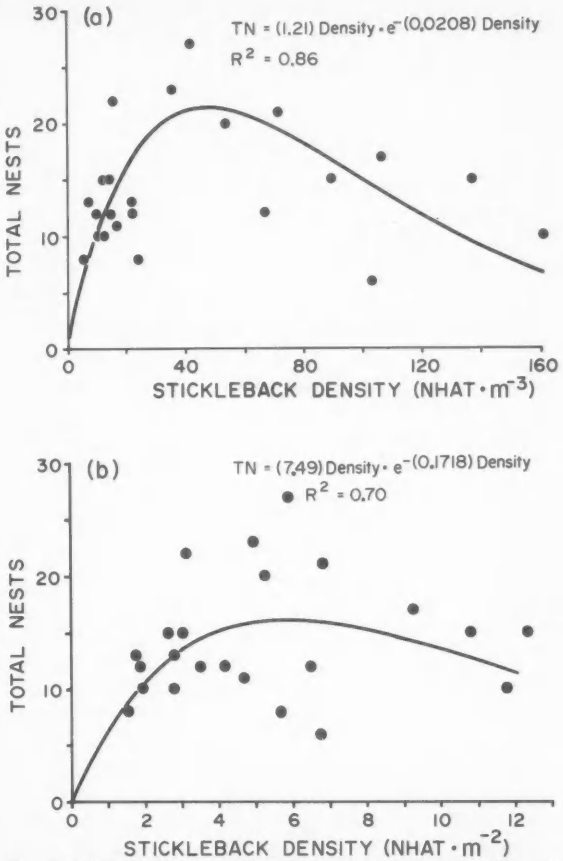

FIG. 7. Relationship between the total number of nests identified and the density of both territorial and nonterritorial sticklebacks maintained in experimental enclosures. Results are presented with density expressed as either (a) fish·m^{-3} of water, or (b) fish·m^{-2} of water surface in enclosures. The solid line represents the Ricker (1975) stock–recruitment function fitted to the data. Parameter estimates are provided in Table 7.

vations indicates a significant increase ($\chi^2 = 7.16$, $df = 2$, $p < 0.01$) in the proportions of sticklebacks classified as egg-eaters in samples obtained between low and intermediate and between low and high onshore abundance levels (Table 5). Similar within- and between-year trends were apparent with respect to the association between variations in onshore abundance of breeding sticklebacks and the number of eggs included in their diets (Fig. 6a, b). Differences in the apparent influence of onshore abundance changes on patterns of egg-eating between 1982 and 1985 may have been a consequence of a failure of the 1985 breeding population to achieve abundance levels equivalent to those observed in 1982 (i.e. both the range and maximum catch for 5-h trap-sets during 1982 were more than double those observed from 24-h trap-sets during 1985).

Nest Density and Survival Rate Experiments

A total of 178 nests were identified in the experimental enclosures from July 11 to August 20, 1985. The number of nests per 9.2–9.7 m^2 enclosure ranged from 6 to 27 (Table 6). Analysis of nesting and agonistic behaviors (Ringler and Hyatt, unpubl. data) revealed no detectable changes in behavior of males within 24 h of enclosure, and no significant differences in behavior attributable to nesting in a particular enclosure. We detected mainly minor changes in density that were presumably associated with movements of fish beneath the wire mesh of the enclosures, and with predation by belted kingfishers (*Megaceryle alcyon*) observed perching nearby. Planned manipulations and observed mortalities accounted for other changes in final population sizes of fish in enclosures (Table 2). Our analysis included all surveys (23 of 28; Table 6) in which more than five nests were identified per enclosure. This reduced error associated with small sample sizes of nests, particularly with regard to estimates of survivorship rate. Subsequent analysis showed that our major conclusions also held based on the entire data set.

The number of nests increased to a peak at intermediate fish density, and decreased at high density (Fig. 7). We used the stock–recruitment function of Ricker (1975) to examine the effect of stickleback population density on nest numbers. Ricker's Equation 11.9 becomes;

$$\text{total nests} = a \cdot \text{Density}\, e^{-B \cdot \text{Density}},$$

where a is a proportionality constant and B measures the strength of effect of population density on nest numbers. Transformation yielded a linear relationship that was fitted by least squares regression.

$$\text{Log}_e \text{ total nests} - \text{Log}_e \text{ Density} = \text{Log}_e a - B \cdot \text{Density}.$$

The slope of the regression provides an estimate of B, and the ordinate intercept is an estimate of $\log_e a$. This model accounted for 70–86% of the variation in nest numbers (Table 7), depending on (a) whether density was expressed per unit area or volume, (b) whether densities were estimated from counts made at the beginning of a week (5–7 d prior to nest identification) or the end of a week (within 1 d of nest identification), and (c) whether nesting males were subtracted from the counts. In all cases the relationship between nest numbers and population density, as depicted by the stock–recruitment model, was statistically significant ($p < 0.0001$). Apriori arguments could be made for employing either of the population counts that we used, but both explained similar amounts of variance in nest numbers. Estimates per unit volume (Fig. 7a) provide better

TABLE 7. Values of $\text{Log}_e a$, b and ANOVA table from application of the Ricker (1975) stock–recruitment function, given different ways of estimating population density. Data are from Table 6; $df = 22$.

Density estimate	$\text{Log}_e a$	SE	β	SE	R^2	F value	P
NHAT/area	2.014	0.150	−0.1718	0.025	0.70	48.52	<0.0001
NHAT/volume	0.1930	0.118	−0.0208	0.002	0.86	125.90	<0.0001
PNOM/area	2.044	0.156	−0.1646	0.022	0.73	57.26	<0.0001
PNOM/volume	0.1787	0.151	−0.0187	0.002	0.81	87.12	<0.0001
(NHAT-total nests)/area	2.739	0.162	−0.2921	0.035	0.77	69.86	<0.0001
(NHAT-total nests)/volume	0.825	0.168	−0.0304	0.003	0.80	83.56	<0.0001

TABLE 8. Values of intercept of Log_e survivorship, slope, and ANOVA table for versions of the survivorship function, based on different estimates of population density. Values for R^2, F, and P are virtually identical to values based on arcsin transformation; $df = 17$.

Density estimator	$\log_e S$	SE	Slope	SE	R^2	F value	$P > F$
NHAT/area	−0.0688	0.124	−0.1058	0.0188	0.66	31.64	0.0001
NHAT/volume	−0.2317	0.078	−0.0081	0.0011	0.78	55.16	0.0001
PNOM/area	−0.1166	0.108	−0.0848	0.0139	0.70	37.39	0.0001
PNOMA/volume	−0.2453	0.070	−0.0067	0.0008	0.81	68.05	0.0001
NHAT-total nests)/area	−0.2335	0.095	−0.1120	0.0188	0.69	35.51	0.0001
(NHAT-total nests)/volume	−0.3127	0.071	−0.0091	0.0012	0.77	53.53	0.0001

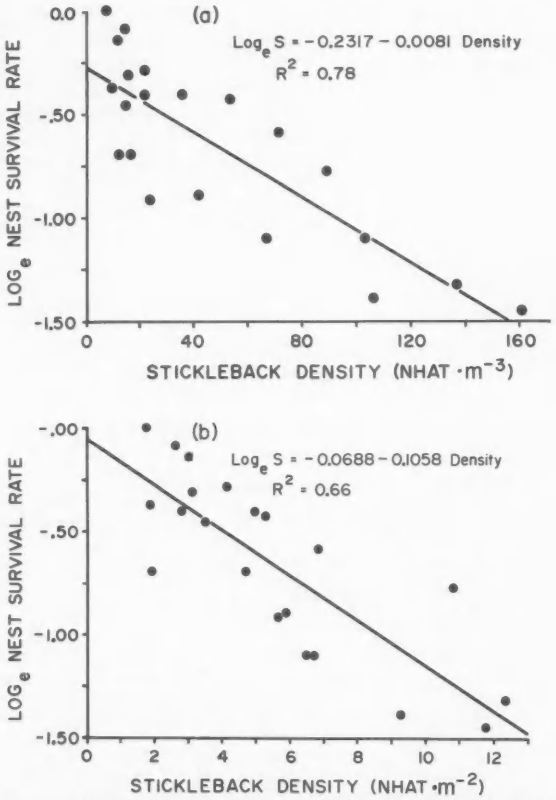

FIG. 8. Relationship between $\log_e$ weekly nest survival rate and the density of both territorial and nonterritorial sticklebacks maintained in experimental enclosures during 1985. Results are presented with density expressed as either (a) fish·m^{-3} of water, or (b) fish·m^{-2} of water surface. The solid lines are the least squares regression fit to the data; see also Table 6.

predictive power, although estimates based on area (Fig. 7b) may be more useful for comparison to other field studies.

Calculations of density for nonnesters were made because behavioral observations suggested that many agonistic encounters occurred between nesting males and both sexes of nonnest-holding fish. Therefore, we hypothesized that density might be better represented by counts in which nesters were eliminated. This estimator was better than one based on total numbers·area^{-1} (Table 7), but poorer than one based on numbers·volume^{-1}. Overall, the total number of fish within an enclosure, expressed in terms of volume or surface area, provided a simple and satisfactory measure of effective population density.

TABLE 9. Comparison of the fecundities of females and the clutch size of males for sticklebacks from Kennedy Lake (this study) and Wapato Lake (Kynard 1978). Mean and standard error of clutch size for Wapato Lake males are approximations only due to use of interpolated values from Kynard's figure 7.

		Kennedy Lake 1982	Wapato Lake 1969–70
Seasonal average (standard error) of fecundity for a 55-m female		61.45 (1.09)	164.2
Average (standard error) of clutch sizes of males	site 1	198.60 (17.36)	
June 20–July 31 from two Kennedy Lake locations and mid-July to early August at Wapato Lake	site 2	286.40 (28.79)	430 (<14.74)

The effects of population density on the number of nests per enclosure could reflect changes in the probability of nests persisting between weeks, changes in the rate at which new nests were established, or both. Weekly estimates of nest survival were used to examine the first possibility. The second was examined by analyzing the number and percentage of "new" nests established weekly. Rates of nest survival ranged from 0.23 to 1.0, and these weekly rates were highly density dependent. The best model of the relationship between survivorship and density (Table 8) employed Log_e survival rate against stickleback density ($r^2 = 0.66$–0.81). This relationship is shown for two of the density estimators in Fig. 8.

A weak ($r^2 = 0.28$) though significant ($p < 0.014$) relationship existed between the proportion of new nests identified and stickleback population density. This finding probably reflects the diminished contribution of "old" nests at high fish density. The number of new nests per stickleback was variable, with an apparent peak of about 0.32 nests·fish^{-1} at densities near 6 fish·m^{-2}. One of the lowest values (0.09 new nests·fish^{-1}) occurred at the highest density, near 12 fish·m^{-2}. One interpretation of this result is that the rate of nest establishment was reduced at high densities. Weekly observations, however, were probably too infrequent to distinguish this effect from that of reduced rates of nest survivorship. Several instances were observed of nests that were established and then lost or moved over the course of 24 h or less.

Discussion

Stickleback Population Regulation

Wootton (1985) reviewed extensive experimental work carried out in the laboratory and outlined a series of hypotheses

concerning the mechanisms involved in the regulation of natural populations of sticklebacks. In Wootton's view, regulation of natural stickleback populations is achieved on the breeding grounds, either through the influence of limited food supplies or of limited breeding space on the ability of breeding males to produce young. He contrasted two extreme sets of conditions at low and high food levels.

At low food levels, the number of breeding males is not limited by the space available in breeding colonies, but rather by food availability, which limits the rate of maturation of males and onshore recruitment from the relatively large pool of males present in the prebreeding population. Therefore at low food levels, low density populations of males with large territories are established throughout the breeding season. Low food levels also depress the total egg supply available on the breeding grounds since low rations favour the development of small, low-fecundity, females with long interclutch intervals. Consequently, the total egg supply will not meet the demand of even low density populations of territorial males. Accordingly, the total recruitment of young as well as the recruitment per male will be low and may be considered to be controlled by the availability of food.

By contrast, Wootton argues that at high food levels most males in the prebreeding population may achieve sexual maturity early in the breeding season; however, the number of territorial males is likely to be limited by the availability of space in breeding colonies. Thus, high density populations of males, occupying relatively small territories, are established throughout the breeding season. Further, high food levels are likely to increase the total egg supply available on the breeding grounds since high rations favour the development of large, high-fecundity, females who display relatively short interclutch intervals. Thus, the total egg supply is likely to exceed the capacity of even a high density population of males to fertilize and care for them. Accordingly, the total recruitment of young will be high and may be considered to be limited by the availability of space which limits the total number of males on the breeding grounds.

Wootton's view of stickleback population regulation does not appear to explain the patterns in the Kennedy Lake population. For example, in Wootton's view, populations may not be food limited and establish high densities of territorial males. However, this combination of traits most closely describes the situation in Kennedy Lake and warrants closer examination.

A number of observations in the present paper, as well as from other studies, suggest that Kennedy Lake sticklebacks are food limited. Kennedy Lake is an ultraoligotrophic lake which, on a global scale, places it close to the bottom of the range for both primary and secondary production levels commonly observed in freshwater (Stockner and Shortreed 1985). Recent field enclosure experiments (O'Neill and Hyatt 1987) have shown that the standing crops and mean size of planktonic prey organisms are depressed by the foraging activities of stickleback populations as large as those observed in the limnetic zone of Kennedy Lake prior to either the 1982 or 1985 breeding seasons (Table 3) and that sticklebacks are actively competing with juvenile sockeye salmon (*Oncorhynchus nerka*) for limited food supplies in the lake. The extent of this food limitation for sticklebacks is evidenced by the small mean size of adults that breed in many areas of the lake (Fig. 2) and maintenance by female sticklebacks, throughout the breeding season, of one of the lowest size-specific fecundities reported for the species to date (only 62 eggs for a 55 mm Kennedy Lake female, versus 164 eggs for a Wapato Lake female of the same size, Table 9).

We concur with Wootton, that low food rations before and during the breeding season permit only a portion of potential breeders to mature at one time. The evidence for this is the dramatic variation in stickleback numbers that recruit onshore throughout the breeding season (Fig. 3). Acoustic and trawl surveys throughout the breeding season confirm that a significant but variable portion of the potential pool of breeding fish continues to occupy offshore waters of the lake. However, although only a portion of the adults engage in breeding activities in onshore locations at any one time, this does not lead to low densities of territorial males as suggested by Wootton. Indeed, densities of territorial males at least as high as those observed in our enclosure experiments, up to $4 \cdot m^{-2}$, were seen in natural breeding colonies both in 1985 and 1982. At these times, competition for space was indicated by the presence of many male fish which were in nuptial coloration but which had no identifiable nests. Thus, although offshore food supplies may control the proportion of the adult population available to recruit onshore, the number of participating adults involved appears more than adequate to saturate the available space.

Some knowledge of the distribution of breeding colonies, observations of the depth distribution of breeding animals, and estimates of the size of the potential breeding population in Kennedy Lake may be used to provide a crude but useful estimate of the densities that stickleback males could achieve in natural colonies during the breeding season. Extensive inspections of shoreline areas during the breeding season in 1982 and in 1985 indicated that steeply plunging profiles and unsuitable substrates (e.g. wave washed rock, coarse sand devoid of cover, vegetation, or food) likely restrict useable breeding areas of Clayoquot Arm to less than one fifth of the shoreline, a distance equivalent to roughly 8.3 km (Rutherford et al. 1986). Breeding males generally establish nests at depths of less than 2 m and, due to the steeply plunging shoreline, this zone will constitute a band of no greater than a 3 m average width. Therefore, there may be roughly 25 000 m^2 of useable breeding area in Clayoquot Arm. Prior to the 1985 breeding season there were 2.94 million sticklebacks in the offshore waters of Clayoquot Arm (Table 3). Given an observed sex ratio of 1:1 in April, we estimate that simultaneous recruitment of all sticklebacks onshore to breed would result in approximately 58 $males \cdot m^{-2}$. Since onshore recruitment to breed is a continuous rather than a simultaneous process (Fig. 3), breeding colony densities this high would not be achieved. Also, both field and laboratory studies suggest that 5–6 $males \cdot m^{-2}$ will saturate the available breeding space in a colony (Stanley 1983; Van den Assem 1967; Black and Wootton 1970; Kynard 1978). Thus, considerable potential exists for the Clayoquot Arm population to saturate onshore locations with males during the breeding season.

In spite of the occurrence of high density populations of males within Kennedy Lake breeding colonies, it appears that, contrary to Wootton's suggestion, recruitment of stickleback young to the population is not directly controlled by space limitations on males within breeding colonies. Observations within two breeding colonies in Kennedy Lake in 1982 indicated that Kennedy males obtained clutch sizes that averaged only 199–286 eggs, by contrast with average clutch sizes of 430 eggs reported by Kynard (1978) for Wapato Lake sticklebacks (Table 9). Kennedy Lake males appear to obtain no more than

46–77% of the eggs that they are capable of fertilizing and caring for during a reproductive cycle. Thus, limited supplies of zooplankton prey offshore and correspondingly low fecundities of Kennedy Lake females appear to be contributing factors limiting recruitment of stickleback young in Kennedy Lake.

The Significance of Nest-Raiding and Egg-Eating

Morris (1952), in a study of ten-spined sticklebacks (*Pygosteus pungitius*), considered egg-eating to be an incidental consequence of pseudofemale behavior on the part of sexually frustrated males precluded by high population density from obtaining either territories or clutches of their own. He interpreted such behavior to be ecologically significant in that variations in breeding population density would induce a greater frequency of pseudofemale activity, which in turn would promote higher levels of egg-stealing and eating (i.e. heterocannibalism). Heterocannibalism was viewed by Morris as having considerable potential to regulate populations.

In contrast, Rohwer (1978) concluded that egg consumption may be largely a consequence of male nestholders consuming a portion of their own eggs. He called this the "filial cannibalism hypothesis" (FCH) which states that guardian males may maximize their reproductive output by eating some of their own genetic offspring as an investment in future reproductive effort. Filial cannibalism is restricted to territorial males guarding nests and, as described by Rohwer, has considerable evolutionary significance as a part of the stickleback male's reproductive strategy but is unlikely to feature prominently in regulation of stickleback populations.

We examined our field evidence in light of these two contrasting interpretations. Two elements of our study argue against filial cannibalism as the predominant source of egg-eating by Kennedy Lake sticklebacks. First, the samples of sticklebacks used for diet analysis in our study were likely composed of predominantly nonterritorial animals since territorial males are only rarely obtained in the types of minnow traps we used for sampling (Worgan and FitzGerald 1981). Therefore, very few of the animals in our samples would qualify as even potential filial cannibals. Second, detailed analysis of stickleback diet data failed to lend support to predictions stemming from Rohwer's FCH and involving identifiable patterns of egg-eating that should be associated with filial cannibalism (Hyatt and Ringler 1989). Thus, it appears that heterocannibalism is the major process shaping the patterns of egg-eating quantified in our study of Kennedy Lake sticklebacks.

Evidence accumulated from observations of both natural and experimental populations of sticklebacks suggests that heterocannibalism may be a potent mechanism for density dependent regulation of interannual variations in stickleback abundance. Whoriskey and FitzGerald (1985b) have recently demonstrated density dependent patterns of egg cannibalism for populations of *G. aculeatus* enclosed within tidal pools and, in accord with their results, we found that the incidence of heterocannibalism increases with increases in the density of breeding sticklebacks. Further, we commonly observed groups of nonterritorial males and females engaging in nest raiding behaviors within natural breeding colonies. The signficance of such ephemeral events may be readily overlooked. A nest raid occurs over periods of seconds to minutes, whereas incubation of embryos and defense of fry requires approximately 13–14 d (Kynard 1978) at the temperatures prevailing during the breeding season in Clayoquot Arm. Thus, even relatively infrequent episodes of nest raiding and egg consumption may have a major impact on nesting success and fry production of territorial males. Our finding of eggs in 11.25% of all fish sampled suggests a relatively high rate of success of conspecifics in invading territories of defending males within natural breeding colonies of Kennedy Lake during 1982 and 1985. This is not to say that the defense tactics of male sticklebacks are ineffective in minimizing nest losses. To the contrary, Whoriskey and FitzGerald (1985a) have shown experimentally that, in the absence of a defending male, nests in tidepool habitats are virtually certain to be raided and destroyed by conspecifics within a 24-h period. However, in spite of such demonstrations of the effectiveness of male nest defense in decreasing the chances of nest loss, we have found that changes in population density within natural breeding colonies were associated with increased heterocannibalism which in turn suggests an influence of population density on nest loss.

Results from our enclosure experiments represent the most compelling evidence that nest raiding, egg predation, and reduction in nesting success are important density-dependent aspects of the littoral phase of the life history of sticklebacks in Clayoquot Arm. The enclosure experiments were designed to represent realistic conditions for stickleback nesting. The experiments were carried out during the major breeding season, following the early seasonal movement of animals inshore, and before the mass migration to overwinter in offshore waters began. An intentional delay in construction and completion of the lakeward wall of the enclosures permitted mature males to initially establish nests along the lakeshore, as they normally would, but within the bounds of the enclosures. The maximum number of nests established in the enclosures ($3 \cdot m^{-2}$) was no higher than that observed in many unenclosed lakeshore locations. Finally, although we did not conduct formal counts of daily variations in the absolute abundance of sticklebacks within breeding colonies, it was obvious that the maximum densities of sticklebacks (nesters and nonnesters) used in the enclosure experiments ($12.3 \cdot m^{-2}$) were lower than the maximum densities frequently achieved during short intervals of observations of sticklebacks in several unenclosed breeding sites in 1985 and especially 1982.

Our analysis of nest survivorship rates from the enclosure experiments indicates that the probability of a male successfully defending his nest (over a 1-wk period) decreases from abut 0.8 at a density of 1 fish$\cdot m^{-2}$ to 0.25 at a density of 10–12 fish$\cdot m^{-2}$. Thus, raids by conspecifics are a threat to nesting success which becomes increasingly more serious as the total abundance of sticklebacks increases within breeding colonies. Territorial males compensate to some extent for density-dependent changes in the risk of heterocannibalism by altering both the frequency and types of defensive behaviours they use (Ringler and Hyatt, unpubl. data). However, the overriding density-dependent effect is clear (Fig. 7 and 8) and it would appear that the twin phenomena of nest raiding and egg cannibalism may constitute a powerful mechanism, in addition to food limitation, for regulating the production of stickleback fry from breeding colonies within Kennedy Lake.

The results of the present study on factors contributing to population regulation of sticklebacks in a coastal lake have practical implications. The Department of Fisheries and Oceans has made a major effort to develop techniques to enhance the production of anadromous and nonanadromous salmonids in a variety of freshwater habitats in British Columbia (Anonymous 1983, 1984). The planting of juvenile coho into freshwater lakes

and streams, which are apparently "barren" of salmonids, and the controlled addition of inorganic nutrients to oligotrophic coastal lakes used for rearing by sockeye salmon, have shown considerable promise as salmonid enhancement techniques. However, application of these techniques is frequently carried out without full knowledge of the role that potential competitors such as threespine sticklebacks might play in reducing the potential benefits that will accrue to the species targeted for enhancement. O'Neill and Hyatt (1987) stressed that the production dynamics of many salmonid and stickleback populations will be interdependent in the low productivity freshwater environments of the outer coast of British Columbia. Accordingly, studies into factors potentially regulating populations of salmonid competitors such as the threespine stickleback, may reveal as much about how to manipulate freshwater habitats to increase the production of commercially valuable salmonids as studies of the salmonids themselves.

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Impact of Diet on Metabolism and Swimming Performance in Juvenile Lake Trout, *Salvelinus namaycush*

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Juvenile lake trout, *Salvelinus namaycush*, of similar size were fed one of three isocaloric diets, each differing in protein and lipid content. Oxygen consumption and swimming performance were measured in a recirculating water flume at intervals throughout the 70-d feeding trials (10°C). Swimming speed was increased by stepwise velocity increments (5 $cm \cdot s^{-1}$) and oxygen consumption was measured at each velocity between 20 and 45 $cm \cdot s^{-1}$. Oxygen consumption for a given speed did not differ significantly throughout the feeding trial nor among the diets implying a similarity in the quality and quantity of substrate catabolized for energy. Basal metabolism (0 $cm \cdot s^{-1}$) was also independent of diet and feeding interval. Critical swimming speed increased with dietary and carcass protein content to suggest a direct association with muscle mass and number of myofilaments.

Des truites fardées juvéniles, *Salvelinus namaycush*, de taille semblable ont été nourries selon l'une de trois diètes isocaloriques, chacune différant selon la teneur en protéines et en lipides. La consommation d'oxygène et le rendement à la nage ont été mesurés dans un canal à recirculation d'eau, à différents intervalles durant les 70 jours d'essais (10°C). La vitesse de la nage a été augmentée par étapes (5 $cm \cdot s^{-1}$) et la consommation d'oxygène a été mesurée à chaque vitesse entre 20 et 45 $cm \cdot s^{-1}$. A une vitesse donnée, la consommation d'oxygène n'a pas varié d'une manière significative pendant la durée de l'essai et n'a pas varié d'une diète à l'autre, ce qui implique une certaine similitude dans la qualité et la quantité du substrat catabolisé en énergie pour la nage. Le métabolisme basal (0 $cm \cdot s^{-1}$) est également resté indépendant de la diète et de l'intervalle entre les repas. La vitesse critique de nage s'était accrue avec l'augmentation de la teneur en protéines de la carcasse, ce qui semble indiquer une association directe entre la masse musculaire et le nombre de myofilaments.

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Maintenance of a living organism requires the continuous expenditure of energy, the actual amount being a manifestation of its many and diverse activities. The irreducible energy cost of maintenance occasioned in fish during rest and in a post absorptive state is represented by the basal metabolic rate (BMR). Swimming is a major consumer of energy in fish and has been classified into three categories, sustained, prolonged, and burst speeds (Beamish 1978). Each reflects not only the constraints imposed by time but also by the biochemical processes which supply the fuel for their application. Energy for BMR and for swimming at sustained and low to intermediate prolonged speeds is derived from aerobic metabolism and can be equated to oxygen consumption (Beamish 1978). At higher swimming speeds anaerobic metabolism becomes increasingly important, complicating the measurement of total metabolism.

Total metabolism depends on both diet and amount of food ingested by fish (NRC 1981). Thus, the provision of adequate energy through dietary lipid can reduce the amount of protein used as an energy source (Watanabe 1977; Reinitz et al. 1978; Takeuchi et al. 1978; Beamish and Medland 1986).

BMR of fish is generally assumed to be independent of diet (NRC 1981) but measurements have not been made. In mammals, a close interspecific relationship between body protein synthesis and BMR has been demonstrated by Waterlow (1968). Substantial protein degradation and synthesis occurs even within cells of fasting and resting animals (Vandenburg and Kaufman 1981; Waterfield et al. 1983). The present study measured the effect of dietary protein and lipid on basal and swimming metabolism and performance of juvenile lake trout (*Salvelinus namycush*).

Materials and Methods

Juvenile lake trout (n = 400; 10–20 g) were obtained from the Ontario provincial fish culture station at Skeleton Lake (Lake Manitou stock; Ihssen et al. 1988) and transported to the laboratory where they were held in a 1100-L circular fiberglass tank continuously supplied with nonchlorinated groundwater. Fish were acclimated to the experimental temperature of 10°C by a daily change of one degree. Chemical composition of the water was not appreciably different from that reported by Hodson and Sprague (1975). Dissolved oxygen and ammonia nitrogen were at all times above and below 9 and 0.1 $mg \cdot L^{-1}$, respectively. Fish were fed to satiation once daily with a diet formulated for salmonids (GRT-7-2, Martin Feed Mills, Elmira, Ontario). Photoperiod was maintained at 16 h with overhead incandescent lights providing an intensity of approximately 60 lx at the surface of the uncovered portion of the holding tank. Light intensity was gradually increased or decreased for 0.5 h at either end of the photoperiod.

TABLE 1. Composition and gross energy of the experimental diets. Nominal values for protein and lipid are in parentheses.

Ingredient	Diet (% weight) 1	2	3
Fish meal (capelin, 68% C.P.[a])	20.0	27.0	35.0
Blood meal	4.0	5.0	7.0
Corn gluten meal	8.0	11.0	14.0
Soybean meal (48% C.P.)	8.0	11.0	14.0
Wheat middlings (15% C.P., 8% fiber)	19.9	11.3	0.3
Wheat middlings, hydrolysed	20.0	20.0	20.0
Vitamin premix[b]	0.5	0.5	0.5
Mineral premix[b]	1.0	1.0	1.0
Fish oil (herring with antioxidant)	18.0	13.0	8.0
Methionine	0.4	—	—
Choline chloride (60%)	0.2	0.2	0.2
Composition			
Crude protein	29.40 (30)	34.65 (35)	43.18 (43)
Lipid	20.90 (20)	17.64 (16)	12.79 (12)
Water	4.63	4.33	5.88
Gross energy (MJ/kg)	22.51	21.59	20.75

[a]C.P. = crude protein.

[b]Refer to Hilton and Slinger (1981) for composition of vitamin and mineral supplements.

Lake trout were maintained in the holding tank for at least 3 wk before transfer to smaller, oval shaped tanks (90 L) supplied with continuous flows of groundwater (10.0 ± 0.5°C). Each tank was equipped with a submersible pump fitted with a perforated vertical tube to assure a water velocity of 20 ± 5 cm·s^{-1} throughout most regions. Fish in each tank were fed one of the three, approximately isocaloric, experimental diets (Table 1) for the prescribed periods of 10, 20, 30, 50, or 70 d before measuring their swimming metabolism. Each tank of fish (n = 20) was fed to satiety once daily. Two tanks of fish were assigned to each combination of diet and feeding period. Feeding schedules were staggered to produce fish of similar size for measuring oxygen consumption.

Swimming metabolism was measured in Blazka-type respirometers (3.2 ± 0.2 L; Waiwood and Beamish 1978). The driving mechanism was a propeller coupled with a submersible pump motor, the speed of which was controlled by a rheostat. The swimming compartment was 20 cm long and 6 cm in diameter. Water velocity was measured by determining the revolutions per minute of a previously calibrated indicator propellor located downstream from the swimming compartment. Revolutions were counted by a stroboscope. Velocity profile in the swimming chamber was approximately rectilinear. Cross sectional area of the fish did not exceed 10% of that for the swimming portion of the chamber, so that no correction was necessary for the volume effect (Smit et al. 1971). Respirometers were submerged in a water bath to assure a constant ambient temperature (10.0 ± 0.1°C).

Oxygen consumption was determined for each of a progression of swimming speeds between 20 and 45 cm·s^{-1}. Prior to the commencement of measurements and 54 h after the final meal in their feeding schedule, fish were transferred to the metabolic chambers and forced to swim against a current of 20 cm·s^{-1} for 16 h. Fish in the respirometers were exposed to continuous light (60–80 lx) since it was found in other species that such a regime reduced diurnal fluctuations in metabolic rate (Hutchinson and Kohl 1971).

An experiment was begun with lake trout swimming against a current of 20 cm·s^{-1} by measuring oxygen concentration of chamber water just before the flow of aerated water was stopped for 45 min. Immediately after the chamber was opened to a fresh supply of aerated water, oxygen concentration was again measured and velocity increased by 5 cm·s^{-1}. The difference in oxygen content between the beginning and end of the 45-min period, minus the aerobic demands of the chamber in the absence of fish provided the measurement of oxygen consumption. After each velocity increment a chamber was alternately opened to a fresh supply of water for 15 min and closed for 45 min. Generally, oxygen concentration in the respirometers did not decline below 80% of air saturation. Following the measurement of oxygen consumption at 45 cm·s^{-1}, fish were removed, weighed and measured to the nearest 0.01 g and 1 mm, respectively.

Basal oxygen consumption was estimated from the linear relationship between the logarithm of oxygen consumption and swimming speed (25–45 cm·s^{-1}). Measurements of oxygen consumption at 20 cm·s^{-1} were deleted from calculations as most lake trout exhibited noticeable spontaneous activity at this speed. The predicted rate of oxygen consumption at 0 cm·s^{-1} was equated to basal when the individual regression correlation coefficient was significant at $p < 0.05$. Oxygen consumption assigned to a fish for each swimming speed was predicted from the regression equation. All rates of oxygen consumption were adjusted to correspond to those for a lake trout of 15 g, the approximate mean weight, using a regression coefficient of 0.85 (Beamish and Dickie 1967). On the relatively few occasions when significance was not demonstrated for an individual regression correlation coefficient, the values for all swimming speeds were discarded.

Swimming performance was measured for lake trout in a 37-L recirculating water tunnel characterized by a uniform velocity profile throughout the swimming compartment (Farmer and Beamish 1969). The experimental protocol was to transfer three fish from the appropriate tank 54 h after their last meal and 16 h before the initiation of a performance test. During the period in the chamber fish were forced to swim against a current of 30 cm·s^{-1}. Thereafter, fish were subjected to a stepwise progression of velocity increments (5 cm·s^{-1}) each for 60 min until fatigued. Aerated groundwater was added continuously to the respirometer, assuring an oxygen concentration at or near air saturation. Time to fatique was recorded for each fish. After the three fish stopped swimming, they were removed and measured to the nearest 1 mm. Critical swimming speed (U_{crit}) was calculated for each fish from the formula described by Brett (1964)

$$U_{crit} = V + (T/t \cdot v),$$

where U_{crit} is critical speed as centimetres per second, t is time increment in minutes, v is velocity increment in (centimetres per second), T is time elapsed at the final velocity in minutes and V is the highest velocity maintained for the prescribed time period as centimetres per second. U_{crit} of each fish was adjusted to the overall mean length of 12.7 cm using the regression coefficient, 0.430, found for juvenile brook trout *Salvelinus fontinalis* (Beamish 1980). An electrical stimulus was not applied to force fish to swim. Conditioning in the holding tanks obviated the need to apply an electrical stimulus.

Proximate composition and energy concentration of lake trout was measured immediately before and 70 d after feeding the respective experimental diets. Trout were killed by an overdose of methane tricaine sulfonate (>100 mg·L^{-1}) and freeze-dried to constant weight in a sublimator. Protein nitrogen content was determined by the micro-Kjeldahl method of Fels and Veatch (1959), protein being estimated from the measurement of nitrogen multiplied by 6.25. Lipid analyses were performed by the wet extraction technique of Folch et al. (1957). The inorganic component of tissue (ash) was represented by the material remaining after heating to 550°C for 24 h as recommended by the Association of Official Analytical Chemists (1965).

Statistical significance was determined by analysis of variance, regression analysis and Duncan's new multiple range test (General Linear Models Procedure, SAS Institute Inc., Cary, North Carolina 1985).

Results

Basal oxygen consumption of lake trout did not change significantly over the 70-d feeding schedule for any of the three diets ($F_{107,5} = 1.39$, $p > 0.05$) nor among the experimental diets ($F_{90,2} = 0.36$, $p > 0.05$). Overall, basal oxygen consumption for a 15-g lake trout was 1.014 ± 0.102 mg O_2·h^{-1} (± 95% C.L., $n = 113$).

The logarithm of oxygen consumption increased linearly with swimming speed irrespective of the diet fed to lake trout. The rate of increase in oxygen consumption with swimming speed did not change significantly throughout the 70-d feeding schedule for any of the diets ($F_{30,5} = 1.76$, 1.88, and 1.89, respectively for diets 1, 2, and 3; $p > 0.05$; Table 2). Furthermore, the rate at which oxygen consumption increased with swimming speed after each of 0, 10, 20, 30, and 50 d of feeding did not differ significantly among the three diets ($F_{12,2} = 0$, 0.36, 0.86, 2.62, and 0.23, respectively for 0, 10, 20, 30, and 50 d; $p > 0.05$). The relationship between oxygen consumption and swimming speed over the initial 50 d of feeding any of the three diets can, therefore, be described by a single regression

$$\log M = -0.028 + 0.017\,S, \qquad (r = 0.999)$$

where M is oxygen consumption for a lake trout of 15 g, mg O_2·h^{-1} and S, swimming speed as centimetres per second.

After 70 d of feeding, significant differences in the rate at which oxygen consumption increased with swimming speed were demonstrable between diets 1 and 2 ($F_{10,1} = 5.61$; $p < 0.05$) and between diets 1 and 3 ($F_{10,1} = 6.36$; $p < 0.05$; Table 2). In contrast, differences between diets 2 and 3 were not significant ($F_{10,1} = 0.07$; $p > 0.05$) after 70 d of feeding. No evidence for a pattern of gradual increase in metabolism was observed over the 70-d feeding interval for lake trout that were fed diet 1. Hence it was presumed the statistically significant increase in oxygen consumption by trout fed diet 1 for 70 d was not of biological significance. With this assumption, the relationship between oxygen consumption and swimming speed throughout 70 d of feeding any of the three diets can be described by the regression

$$\log M = -0.007 + 0.017\,S, \qquad (r = 0.999)$$

Critical swimming speeds (U_{crit}) differed for trout fed the three diets after both 30 and 70 d of feeding (Table 3). Significant differences in U_{crit} were demonstrable between lake trout fed diets 1 and 3 ($F_{10,1} = 20.42$ and 53.99, respectively for 30 and 70 d, $p < 0.05$). U_{crit} also differed significantly for trout fed diets 2 and 3 ($F_{10,1} = 28.68$ and 38.81, respectively for 30 and 70 d, $p < 0.05$). Lake trout fed diets 1 and 2 had significantly different U_{crit} between 0 and 70 d ($F_{10,1} = 23.77$ and 27.41, respectively for diets 1 and 2, $p < 0.05$) and between 30 and 70 d ($F_{10,1} = 17.68$ and 26.62, respectively for diets 1 and 2, $p < 0.05$). For lake trout fed diet 3, U_{crit} increased significantly from 0 to 30 d ($F_{16,1} = 29.58$, $p < 0.05$), 0 to 70 d ($F_{16,1} = 140.97$, $p < 0.05$) and from 30 to 70 d ($F_{10,1} = 39.67$, $p < 0.05$).

Carcass protein concentration was higher for lake trout receiving the maintenance diet GRT-7-2) than for those fed the experimental diets (Table 4). However, protein concentration differed significantly only between trout fed diets 1 and GRT-7-2 ($F_{8,1} = 5.72$, $p < 0.05$). Carcass lipid concentration was highest and lowest for trout fed diets 1 and 3, respectively. Difference were significant only between diets 1 and 3 ($F_{8,1} = 6.13$, $p < 0.05$) and between diets 1 and GRT-7-2 ($F_{8,1} = 6.70$, $p < 0.05$). Relative water content was significantly lower for lake trout fed diet 1 than diet 2 ($F_{8,1} = 5.67$, $p < 0.05$). Other significant differences in water concentration were not found. Ash concentrations of trout fed diets 1 and 2 were significantly different from that for fish fed the GRT-7-2 diet ($F_{8,1} = 6.06$ and 7.10, respectively, $p < 0.05$).

Discussion

The aerobic fuel for metabolism in feeding fish is drawn from carbohydrates, lipids, and protein but information on the preferred substrate is scarce and inconsistent among species. Kutty (1968) suggested carbohydrates were preferentially used by rainbow trout, *Salmo gairdneri*, at sustained and prolonged swimming speeds. Total body lipid of coho salmon, *Oncorhynchus kisutch*, decreased in direct proportion to distance travelled (Krueger et al. 1968). In exercised *Tilapia mossambica*, fasted for only a short time, the amount of ammonia excreted

TABLE 2. Influence of diet (Table 1) and feeding period on the relationship; logarithm of oxygen consumption (mg O_2·15 g^{-1}·h^{-1}) = $a + b$ · swimming speed (cm·s^{-1}). Correlation coefficients of all regressions were significant at $p < 0.05$.

Feeding period (d)	Diet 1			Diet 2			Diet 3		
	Number of fish	Intercept a	Slope b	Number of fish	Intercept a	Slope b	Number of fish	Intercept a	Slope b
0	6	0.0152	0.0191	6	0.0152	0.0191	6	0.0152	0.0191
10	6	−0.0434	0.0157	7	0.0609	0.0148	6	0.1135	0.0142
20	6	−0.1068	0.0192	5	−0.0837	0.0165	6	0.0253	0.0151
30	5	−0.0127	0.0201	7	−0.0805	0.0174	9	0.0170	0.0157
50	8	−0.2413	0.0217	9	0.0100	0.0155	8	−0.0982	0.0180
70	7	0.1487	0.0169	9	0.0115	0.0152	9	0.0234	0.0146

TABLE 3. Critical swimming speed (U_{crit}) and mean length of lake trout before (0 d) and after feeding (30 and 70 d) the experimental diets. Each value of U_{crit} represents the mean ± 95% C.L. of six fish, adjusted to correspond to that for a lake trout of 12.7 cm (see text).

	Diet 1		Diet 2		Diet 3	
Time (d)	Length (cm)	U_{crit} ($cm \cdot s^{-1}$)	Length (cm)	U_{crit} ($cm \cdot s^{-1}$)	Length (cm)	U_{crit} ($cm \cdot s^{-1}$)
0	12.2±0.4	76.5±2.3	12.2±0.4	76.5±2.3	12.2±0.4	76.5±2.3
30	13.2±0.4	69.6±7.1	12.2±0.8	73.9±4.7	12.8±0.3	84.1±3.1
70	12.6±0.4	83.5±2.8	12.9±0.4	83.6±3.3	12.8±0.7	95.4±3.4

TABLE 4. Proximate composition (percent live weight) of lake trout fed the maintenance diet and after 70 d of feeding the experimental diets. Each value represents the mean ± 95% C.L. of five lake trout.

		Experimental diets		
Component	Maintenance GRT-7-2	1	2	3
Water	75.49±0.51	74.29±1.47	75.72±0.79	75.49±0.51
Protein	18.07±5.86	12.68±3.65	14.17±2.69	15.44±3.42
Lipid	5.57±1.88	8.23±2.49	6.87±1.46	5.65±1.93
Ash	2.67±0.57	2.12±0.26	2.11±0.13	2.44±0.40
Total	101.80	97.32	98.87	99.02

relative to oxygen consumed was sufficiently high to implicate protein catabolism in providing aerobic energy (Kutty 1972). In the present study, oxygen consumption of lake trout at specific sustained and prolonged speeds was independent of carcass proximate composition implying a similarity in the quality and quantity of substrate catabolized for energy. In contrast, under conditions of an extended fast or dietary deficiency, basal and swimming metabolism can be expected to change. During extended periods of food deprivation, fish preferentially catabolize protein and the free amino acid pool over glycogen and lipid (Creach and Serfaty 1974). In accord, BMR of white suckers, *Catostomus commersoni*, and brook trout, declined precipitously during the first few days of food deprivation and thereafter changed little over periods up to 25 d (Beamish 1964).

The protein and lipid concentrations of the three diets fed to the lake trout in the present study are within the range customarily fed to salmonids (DeLong et al. 1958; Slinger et al. 1977; Reinitz et al. 1978; Takeuchi et al. 1978; Cowey 1979). Weight gain in juvenile rainbow trout fed balanced practical diets was independent of protein concentration between 35 and 45% (Slinger et al. 1977). Dietary lipid concentrations between 10 and 20% produced good weight gain in rainbow trout (Lee and Putnam 1973; Takeuchi et al. 1978). Protein was used more efficiently at dietary lipid levels above 10%. Takeuchi et al. (1978) suggested that for rainbow trout, optimum levels of dietary protein and lipid are 35 and 15–20%, respectively, similar to those in diet 2 in this study.

BMR determined for lake trout in this study, 1.014 mg $O_2 \cdot 15\ g^{-1} \cdot h^{-1}$, was similar to that measured earlier by Gibson and Fry (1954), also at 10°C, 0.783 mg $O_2 \cdot 15\ g^{-1} \cdot h^{-1}$. A much higher value for BMR of lake trout, 2.349 mg $O_2 \cdot 15\ g^{-1} \cdot h^{-1}$, was predicted from a regression relating metabolism, body weight, temperature, and swimming speed (Stewart et al. 1983). Further, their regression predicted higher oxygen consumptions for swimming than were found in this study. However, the rate of increase in oxygen consumption with swimming speed was higher in the present study so that at 45 $cm \cdot s^{-1}$, the two measures of metabolism were not far apart at 5.728 and 6.670 mg $O_2 \cdot 15\ g^{-1} \cdot h^{-1}$.

Critical swimming speed varied directly with dietary and body protein and inversely with dietary and body lipid particularly after 30d feeding. After 70d feeding, U_{crit} was highest for lake trout fed experimental diet 3 which had the highest protein concentration whereas U_{crit} for fish fed the other two diets was about the same. Body weight in relation to length did not differ noticeably among lake trout fed the three diets so that the variation in performance is not easily explained on the basis of workload. Synonymous with a rise in body protein is an increase in muscle mass and number of myofilaments. The significance of this to a swimming fish is an improvement in force per stride and the capacity to achieve faster speeds (Blake 1983).

Acknowledgments

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Metal Regulation, Tolerance, and Body Burdens in the Larvae of the Genus *Chironomus*

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Krantzberg, G., and P. M. Stokes. 1989. Metal regulation, tolerance, and body burdens in larvae of the genus *Chironomus*. Can. J. Fish. Aquat. Sci. 46: 389–398.

Bioaccumulation of metals are determined and compared for two populations of chironomid larvae collected from acidic lakes differing in their metal contamination. Results from laboratory experiments indicated that chironomid larvae from the more contaminated lake were able to regulate, or control their accumulation of Cu, Ni, and to some extent, Mn. Both populations regulated Zn, and neither population could regulate Pb or Cd. When larvae from the less contaminated site were exposed to sediments from the more contaminated lake, their growth was retarded and metals were accumulated to higher concentrations than for the native population. The data indicate that chronic exposure to elevated metal levels may result in the development of metal tolerance.

La bioaccumulation de métaux a été comparée dans deux populations de larves de chironomidés échantillonnés dans des lacs acidifiés qui n'étaient pas contaminés par les métaux dans la même mesure. Les résultats de travaux en laboratoire ont indiqué que les larves de chironomidés provenant du lac le plus contaminé pouvaient réguler ou contrôler l'accumulation de Cu, de Ni ainsi que de Mn dans une certaine mesure. Les deux populations parvenaient à réguler la concentration de Zn et aucune ne pouvait réguler celle du Pb ou du Cd. Lorsque des larves provenant du lac le moins contaminé étaient exposées à des sédiments du lac le plus contaminé, on observait que leur croissance était retardée et que les métaux étaient accumulés en concentrations supérieures à ce qu'ils l'étaient dans la population indigène. Les résultats indiquent que l'exposition chronique à des métaux en concentrations élevées peut conduire au développement d'un mécanisme de tolérance des métaux.

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Based on experimental evidence including LC_{90}'s, avoidance, and growth retardation, Wentsel et al. (1978) demonstrated that midge larvae, *Chironomus tentans*, were more resistant to metal contaminated sediments when collected from a site that had received high Cd, Zn, and Cr loadings than were organisms that originated in an uncontaminated basin. Body burdens of metals were not assessed.

Metal tolerance clearly is a frequent occurrence for invertebrates from contaminated environments and, in general, invertebrates tend to regulate and thereby control their accumulation of Zn (Frazier and George 1983; Bryan et al. 1977; Anderson et al. 1978; Icely and Nott 1980). Tolerance to Cu is achieved either through regulation or internal storage in a detoxified form, and may result in higher or lower Cu concentrations in tolerant compared with nontolerant populations (Amiard-Triquet et al. 1983; Saliba and Krzyz 1976). Giesy et al. (1980) noted that the behavior of essential metals, such as Zn, tends to decouple tissue concentrations of such elements from environmental concentrations, thereby eliminating the possible use of many organisms as biomonitors of required metals. Lead and Cd tolerances exist, but are less frequently associated with the capability of regulation and/or elimination than is Zn, and some organisms can maintain constant concentrations of Mn and Fe.

In the present study we examined metal accumulation by two populations of larval *Chironomus* exposed to different sediment types. Although species–specific differences in metal metabolism can contribute to the differences in metal bioaccumulation among *Chironomus* populations, intraspecies variability has been demonstrated to be as significant as interspecies variability for several taxa (Boyden 1977). By examining metal dynamics for the genus *Chironomus*, we attempted to identify some of the factors that influence bioaccumulation sufficiently to override species–specific variation. In addition, if chironomids are to be used as biological monitors of metal availability, speciation of the larvae is not only technically demanding, but is in some cases impossible. Therefore, examining the genus as a whole was deemed appropriate.

Our primary objective, then, was to determine whether metal accumulation by the two populations would differ as a result of their history of exposure to environments that varied in level of metal contamination. Chironomids from a highly contaminated site could potentially develop metal tolerance. If tolerance is due to changes in metal storage, regulation, and excretion, the mechanisms of trace metal accumulation could differ between populations exposed to contaminated and uncontaminated sediments.

In addition, we monitored the initial stages of metal uptake or loss since these would more likely reflect adsorption or desorption of metals bound externally to the epicuticle while the later stages of metal accumulation may be subject to met-

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abolic constraints such as active uptake or elimination. In cases where tolerance is associated with the ability to regulate metals, the rates of metal accumulation and tissue accumulation should be coupled. For example, during growth, metal concentrations may remain constant while total body burdens increase. Differences in the extent of metal accumulation between populations were examined to identify differences in metal metabolism.

Materials and Methods

Site Description

Plastic Lake is a softwater, acidic (pH 5.4 – 5.6), oligotrophic lake located in the Muskoka–Haliburton region of south central Ontario. The lake referred to as Lake 240 (L240W) is also soft, acidic (pH 5.1), and oligotrophic and is located in the Wanapetei region of central Ontario. Because of its proximity to Sudbury, L240W receives higher atmospheric loadings of acid and metals than Plastic Lake. These loadings probably have been high since 1890 (Ontario Ministry of the Environment 1982). Given the resulting elevated metal levels in L240W, the population in L240W could have acquired tolerance to some metals over evolutionary time. Sites were selected in Plastic Lake and L240W such that the physical properties of the sediments were comparable. Data on some physical and chemical lake characteristics are provided by Krantzberg (1987).

Preparation of Culture Collections

To set up experiments in the laboratory, larvae, sediments, and water were collected from the two study lakes. Chironomids were sampled by Ekman grab at 15 m from both Plastic Lake and L240W. Cultures were assembled in the laboratory by placing 2 L of whole sediment (depth of 10 cm), into 7-L polyethylene basins, and adding 5 L of lake water. Approximately 200 chironomids from the same lake as the sediments and water were placed in each basin. The basins were aerated and maintained in complete darkness at 6°C in incubation chambers. Fourth instar larvae were removed as required for the experiments by sieving the sediments through a 200-μm "Nytex" nylon screen.

Experimental Treatments

Experimental systems consisted of simple microcosms with 50 mL (2 cm depth) of sediment and 200 mL of lake water in 250-mL polyethylene beakers. For all experimental treatments the containers were also aerated and maintained in a temperature controlled chamber at 10°C.

Experiment 1: Exposure to Spiked Sediments

To determine whether Plastic Lake and L240W chironomids differed in their response to sediments that were artificially enriched with trace metals, Plastic Lake sediments were spiked with Cu, Zn, Ni, Pb, and Cd to bring total concentrations in whole sediments to approximately 100, 300, 100, 200, and 10 $\mu g \cdot g^{-1}$ dry weight, respectively. The spiking solutions consisted of $CuSO_4$, $ZnSO_4$, $NiCl_2$, $Pb(NO_3)_2$, and $CdCl_2$ salts dissolved in distilled water at a dosing volume of 1 mL of spike solution per 100 mL of sediment. Sediments were thoroughly homogenized for 30 min using a polyethylene spatula and were then placed in polyethylene beakers. Six beakers each of spiked Plastic Lake and unspiked Plastic Lake sediments were assembled and 50 Plastic Lake or L240W chironomids, respectively, were added to each beaker after allowing the beakers to equilibrate for 1 wk. After 72 h and 2 wk exposure at 10°C, the contents of triplicate beakers of each treatment were sieved through a "Nytex" nylon mesh. Larvae that were removed from the sediments were held in lake water for 24 h to evacuate their gut contents, and chironomids were digested in 3 mL analytical grade HNO_3 at 120°C for 24 h.

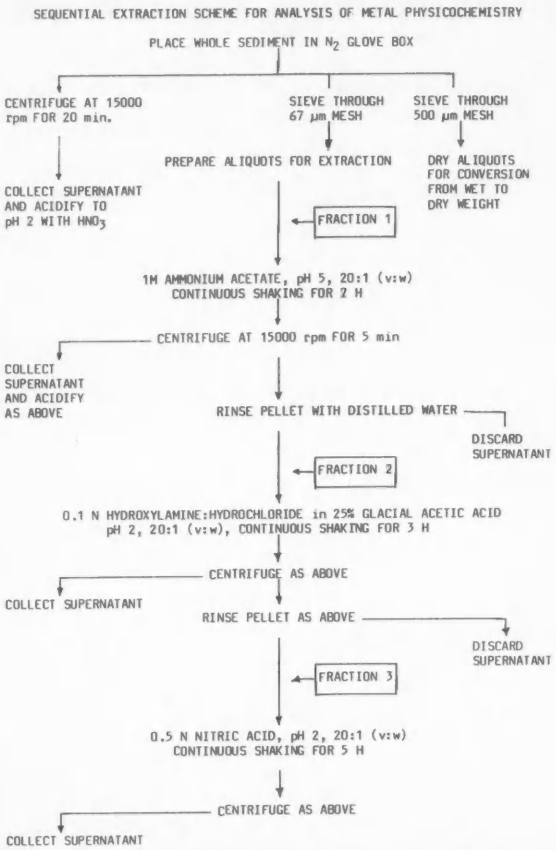
SEQUENTIAL EXTRACTION SCHEME FOR ANALYSIS OF METAL PHYSICOCHEMISTRY
PLACE WHOLE SEDIMENT IN N_2 GLOVE BOX
CENTRIFUGE AT 15000 rpm FOR 20 min.
SIEVE THROUGH 67 μm MESH
SIEVE THROUGH 500 μm MESH
PREPARE ALIQUOTS FOR EXTRACTION
DRY ALIQUOTS FOR CONVERSION FROM WET TO DRY WEIGHT
COLLECT SUPERNATANT AND ACIDIFY TO pH 2 WITH HNO_3
FRACTION 1
1M AMMONIUM ACETATE, pH 5, 20:1 (v:w) CONTINUOUS SHAKING FOR 2 H
CENTRIFUGE AT 15000 rpm FOR 5 min
COLLECT SUPERNATANT AND ACIDIFY AS ABOVE
RINSE PELLET WITH DISTILLED WATER
DISCARD SUPERNATANT
FRACTION 2
0.1 N HYDROXYLAMINE:HYDROCHLORIDE in 25% GLACIAL ACETIC ACID pH 2, 20:1 (v:w), CONTINUOUS SHAKING FOR 3 H
CENTRIFUGE AS ABOVE
COLLECT SUPERNATANT
RINSE PELLET AS ABOVE
DISCARD SUPERNATANT
FRACTION 3
0.5 N NITRIC ACID, pH 2, 20:1 (v:w) CONTINUOUS SHAKING FOR 5 H
CENTRIFUGE AS ABOVE
COLLECT SUPERNATANT

FIG. 1. Sequential extraction scheme for metal partitioning among physicochemical forms.

Experiment 2: Reciprocal Transplant

If L240W chironomids were more tolerant of elevated metals than Plastic Lake larvae, then metal accumulation by Plastic Lake chironomids could differ from L240W larvae when both were held in L240W sediments. The L240W larvae transplanted to Plastic Lake sediments would demonstrate metal elimination or depuration if their body burdens decreased. To examine these hypotheses, we placed 50 Plastic Lake fourth instar chironomids in L240W sediments and 50 L240W larvae in Plastic Lake sediments. Chironomids were also held in their native sediments and harvested concurrently with transplanted larvae to control for changes in body burdens not associated with exposure to a different sediment type. Sixty beakers were assembled and the entire contents of triplicate beakers were harvested after 3 d, 1 wk, 2 wk, 8 wk, and 16 wk. At the time of sampling, larvae were placed in lake water for 24 h to evac-

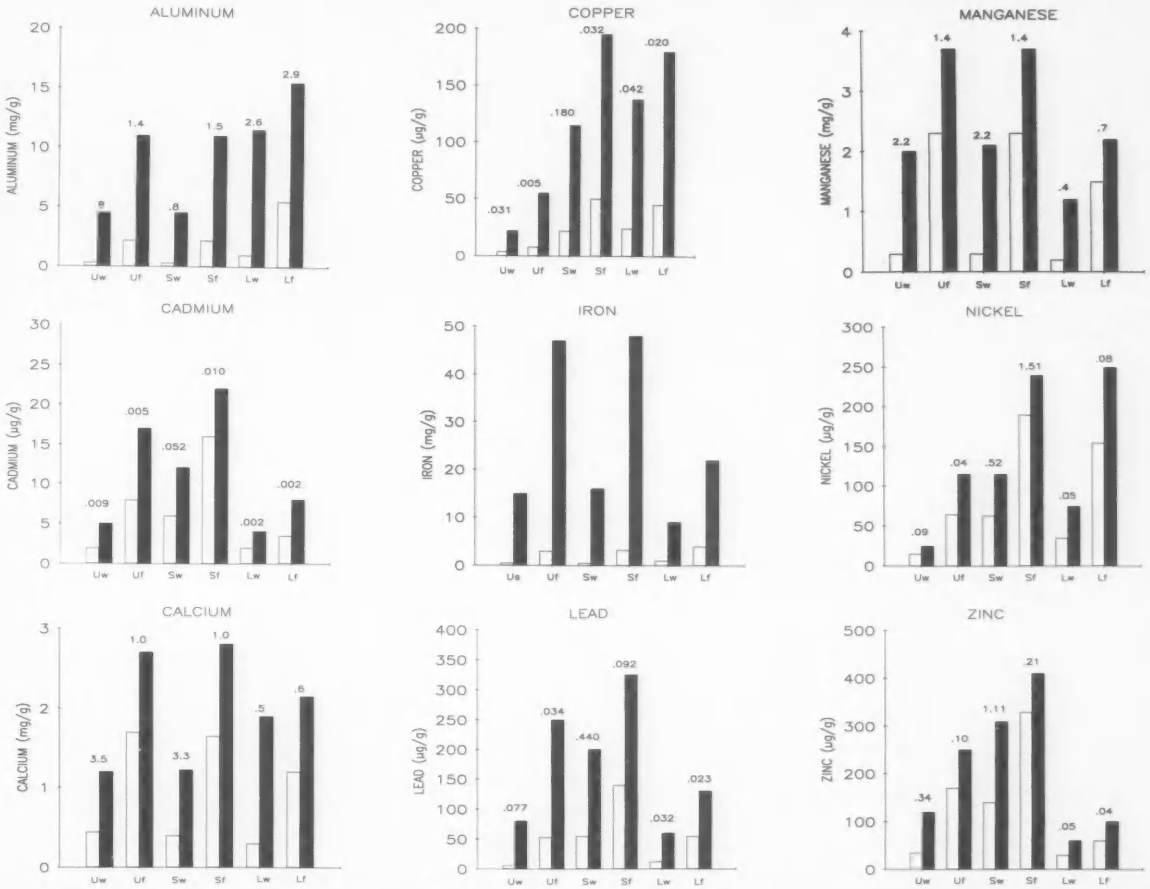

FIG. 2. Metal partitioning in Plastic Lake and L240W sediments. Open bars represent extractable metals and solid bars represent total metal concentrations. *U* refers to unspiked Plastic Lake sediments, *S* refers to spiked Plastic Lake sediments, and *L* refers to L240W sediments. *w* indicates whole sediments while *f* indicates the <67 µm size fraction. Values above histograms represent the MeE/FeE ratios (see text). Sediment concentrations are presented on a dry weight basis.

uate their gut contents, and were then dried on polyethylene weighing boats at 60°C to constant weight and digested in analytical grade, concentrated hot HNO_3 for 24 h. Changes in dry weight per individual through time were also monitored to assess growth.

Sediment analysis

All sediments were subjected to a sequential chemical extraction procedure to operationally define the nature of the metal associations (Krantzberg and Stokes 1985). By treating sediments with different chemical extractants at various pH and temperature regimes (Fig. 1), metals that were extracted could be classified as having been bound to sediments according to the physicochemical nature of the leaching operation. Given that metal redistribution was unavoidable during the course of this procedure and that the precise action of each treatment could only be estimated, the physicochemical forms are said to be operationally defined.

As an additional means of comparing the potential biological availability of sediment-bound metals, we calculated the sum of the extractable metals and normalized these values across the sediment types for Fe in the extractable fractions. The theoretical basis of this procedure is the ability of Fe hydrous oxides to coprecipitate metals and render them less available for uptake by the biota (Tessier et al. 1984; Luoma and Jenne 1977). The parameter was expressed as MeE/FeE, where MeE is the sum of the concentrations of a given metal in the extractable phases, and FeE is the concentration of extractable Fe (Fig. 2).

Acid digested larvae and acidified sediment extracts were analyzed for Al, Mn, Ca, Zn, Ni, Fe, and Cu by an ARL 34000 Inductively Coupled Plasma Argon Emission Spectrophotometer, and for Pb and Cd by a Scintrex Zeeman modulated tungsten filament Atomic Adsorption Spectrophotometer (AAZ-2). National Bureau of Standards oyster tissue and river sediments, and National Research Council lobster hepatopancreas were used as checks on the accuracy of the determinations. Recoveries were within 7% of certified values.

The results of exposure of larvae to different sediment treatments were compared by linear regression analysis and analysis of variance (ANOVA). To satisfy the assumptions of ANOVA, metals in chironomids were log-transformed to achieve a normal distribution of the data set.

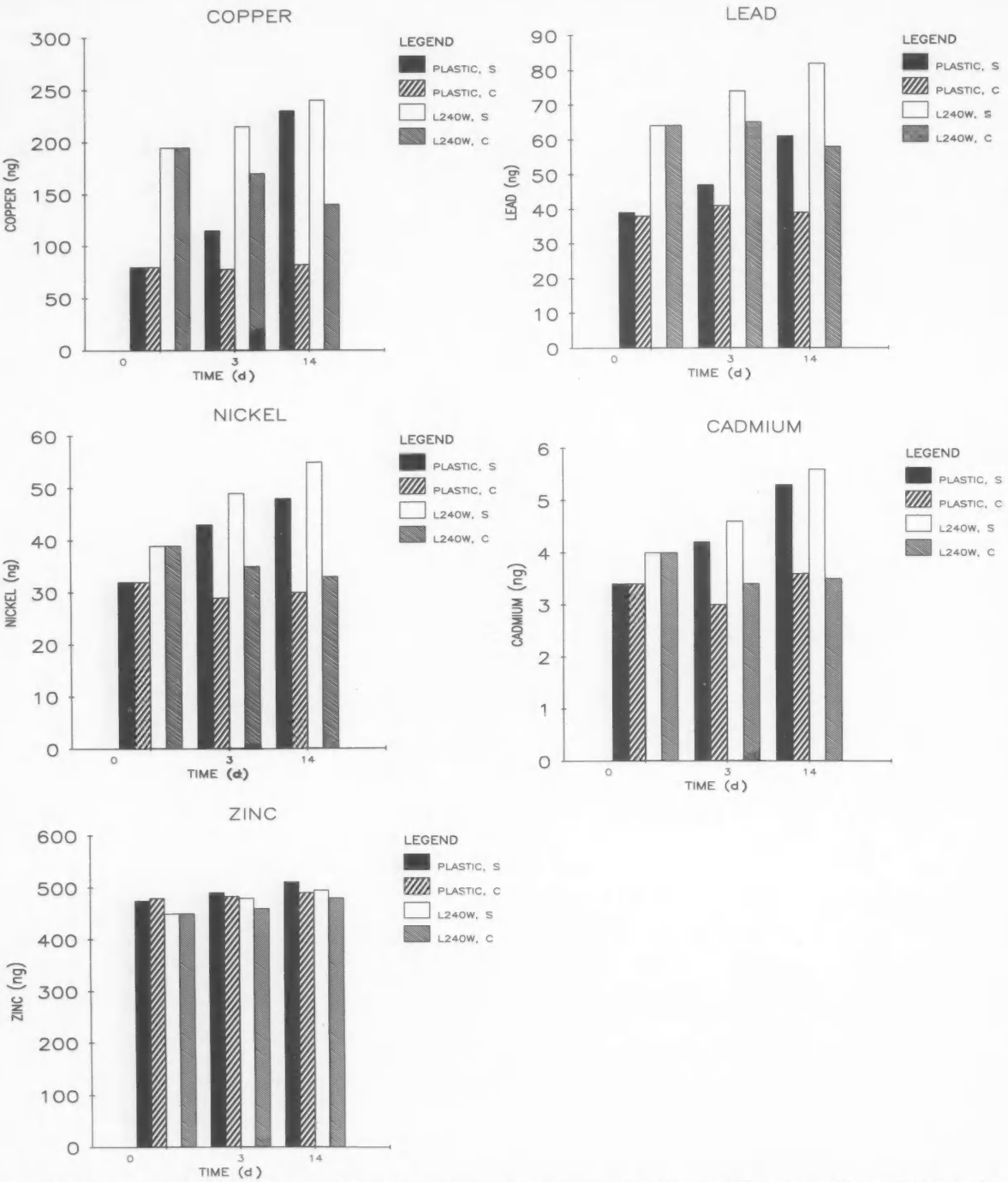

FIG. 3. Metal accumulation by Plastic Lake and L240W larvae in (*S*) spiked and unspiked or (*C*) control Plastic Lake sediments. Values are in nanograms per individual, dry weight.

Results

Experiment 1: Metal Bioaccumulation in Spiked and Unspiked Sediments

Levels of metals in sediments

In general, the greatest amounts of extractable Cd, Cu, Ni, Pb, and Zn were in spiked, Plastic Lake sediments. Unspiked L240W had intermediate levels of extractable metals and the

TABLE 1. Effects on metal body burdens (BB) in chironomids in their own sediments (controls) and transplanted to sediments of different physicochemical characteristics. + indicates the body burdens increased significantly ($p < 0.05$), − indicates a significant decrease ($p < 0.05$), and 0 indicates no significant change ($p > 0.05$).

Population	Sediment	Initial BB	BB on day 3	BB on day 14	Initial effect on BB	Long-term effect on BB
			Lead			
L240W	L240W	0.28	0.28	0.32	0	+
L240W	Plastic Lake	0.28	0.32	0.32	+	0
PL	Plastic Lake	0.14	0.14	0.18	0	+
PL	L240W	0.14	0.13	0.15	−	+
			Cadmium			
L240W	L240W	0.031	0.031	0.038	0	+
L240W	Plastic Lake	0.031	0.036	0.044	+	+
PL	Plastic Lake	0.032	0.032	0.036	0	+
PL	L240W	0.032	0.028	0.031	−	+
			Aluminum			
L240W	L240W	46	47	50	+	+
L240W	Plastic Lake	46	50	54	+	+
PL	Plastic Lake	50	50	55	0	+
PL	L240W	50	49	51	−	+
			Calcium			
L240W	L240W	81	84	90	+	+
L240W	Plastic Lake	81	78	85	−	+
PL	Plastic Lake	78	78	85	0	+
PL	L240W	78	78	80	0	+
			Iron			
L240W	L240W	86	89	98	+	+
L240W	Plastic Lake	86	84	90	−	+
PL	Plastic Lake	38	37	42	0	+
PL	L240W	38	42	48	+	+
			Manganese			
L240W	L240W	2.6	2.6	2.9	0	+
L240W	Plastic Lake	2.6	2.7	2.5	+	−
PL	Plastic Lake	1.1	1.0	1.2	−	+
PL	L240W	1.1	1.2	1.4	+	+
			Zinc			
L240W	L240W	6.3	6.4	6.8	+	+
L240W	Plastic Lake	6.3	6.6	7.1	+	+
PL	Plastic Lake	6.5	6.6	7.2	+	+
PL	L240W	6.5	6.4	6.6	−	+
			Nickel			
L240W	L240W	0.49	0.49	0.55	0	+
L240W	Plastic Lake	0.49	0.47	0.43	−	−
PL	Plastic Lake	0.29	0.26	0.36	−	+
PL	L240W	0.29	0.29	0.48	0	+
			Copper			
L240W	L240W	3.4	3.4	3.8	0	+
L240W	Plastic Lake	3.4	3.3	3.0	−	−
PL	Plastic Lake	1.2	1.2	1.3	0	+
PL	L240W	1.2	1.3	1.7	+	+

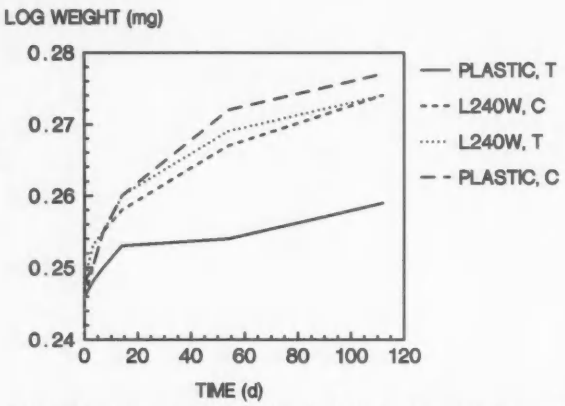

FIG. 4. Changes in biomass of fourth instar larvae exposed to two sediment types. Plastic, *C*, and Plastic, *T*, refer to Plastic Lake larvae exposed to Plastic Lake and L240W sediments, respectively. L240W, *C*, and L240W, *T*, refer to L240W larvae exposed to L240W sediments and transplanted to Plastic Lake sediments, respectively.

lowest amounts were in unspiked Plastic Lake sediments (Fig. 2). Assuming that the ease of extraction of a particular metal serves as a measure of potential biological availability, the bioavailability of any given element is likely to be higher in sediments where more metal was extractable. Therefore, we would predict that metal bioaccumulation would be greatest for organisms exposed to spiked sediments. The MeE/FeE ratios are also presented in Fig. 2. As this value increases, so does the likelihood that metals will be bioavailable.

Lead and Cadmium in larvae

Lead and Cd burdens were significantly greater in Plastic Lake and L240W larvae from spiked sediments than in those from unspiked sediments, regardless of the origin of the larvae. This paralleled the finding that more Pb and Cd were in extractable forms in spiked as compared with unspiked sediments. The rate of accumulation did not differ between larvae from the two populations. When L240W larvae were held in unspiked sediment their Pb and Cd burdens decreased; Plastic Lake larvae in unspiked sediments did not change their levels of Pb and Cd.

Zinc in larvae

At the onset of the experiment, Zn content in larvae from both populations did not differ significantly (ANOVA, $p > 0.05$). The increase in Zn body burdens during the first 3 d was greater in larvae colonizing spiked than unspiked sediments (ANOVA, $p < 0.05$). During the remainder of the experimental period Zn burdens increased, but no significant changes in Zn concentrations were observed for any larvae, regardless of sediment treatment or population origin (Fig. 3).

Copper and Nickel in larvae

For both populations of chironomids, Cu and Ni body burdens increased when larvae were exposed to spiked Plastic Lake sediments and the rates of metal accumulation in the first 3 d were significantly greater than subsequent rates of accumulation ($p < 0.05$). Metal accumulation was significantly greater ($p < 0.01$) for Plastic Lake larvae in spiked sediments than for L240W larvae in the same sediments, indicating that the populations differed in their ability to bioaccumulate these essential trace elements. The total concentration of Cu in spiked sediments was approximately 5 × that of unspiked Plastic Lake sediments, while the extractable concentration was 4–7.7 × that of Plastic Lake (Fig. 2). Nevertheless, Cu burdens of L240W larvae at the onset of the experiment were only 2.5 × those of Plastic Lake larvae. Similarly, the elevated Ni levels in L240W sediments did not result in proportional increases in Ni burdens in L240W chironomids. In addition, L240W chironomids that were held in control sediments decreased their Ni body burdens significantly.

Experiment 2: Concentrations and Whole Body Burdens in Controls Versus Transplanted Larvae

Larvae in their own sediments (controls)

For control larvae that were held in their native sediments during the experimental period, Al, Pb, Mn, Zn, Ni, and Cu contents increased, but concentrations did not change significantly with time, while Fe, Ca, and Cd contents and concentrations increased (Table 1).

L240W larvae transplanted to Plastic Lake sediments

Growth rates for L240W larvae, as measured by net changes in biomass through time, did not differ from their controls (Fig. 4). In some instances, differences in metal accumulation between control and transplanted larvae were restricted to the early phase of the experiment, and subsequent changes in concentrations mirrored the changes in control larvae. For example, Pb concentrations in control larvae increased with time. Lead concentrations in transplanted larvae increased initially, then decreased with time (Table 1). Aluminum and Cd concentrations increased initially in transplanted L240W larvae, but subsequent rates of accumulation did not differ from controls. These patterns may reflect the similarity in binding forms of Al and Cd in Plastic Lake and L240W sediments, based on chemical extraction (Fig. 3). Manganese, Cu, and Ni concentrations gradually decreased in transplanted chironomids in contrast to controls, where concentrations increased, mirroring the greater concentrations and extractability of these elements in L240W sediments (Table 1). Within the first 3 d, Ca concentrations in L240W transplanted larvae decreased, but this was not observed for L240W controls. Subsequent rates of Ca accumulation by transplanted larvae were comparable with controls. Zinc concentrations increased initially for transplanted larvae, but remained constant for the remainder of the experiment despite the greater concentrations of total and extractable Zn in Plastic Lake as compared with L240W sediments. Zinc concentrations in controls were unchanged. Initially, L240W chironomids lost Fe when held in Plastic Lake sediments, and subsequently their Fe concentrations increased more slowly than L240W controls.

Plastic Lake larvae transplanted to L240W sediments

Plastic Lake larvae transplanted to L240W sediments with higher concentrations of some metals showed growth retardation and increased their biomass more slowly than control larvae (Fig. 4). As a result, the amount and direction of change in metal concentrations and whole body content differed for some elements (Table 1). Total Pb burdens in Plastic Lake larvae increased to a greater extent in controls than in transplanted individuals. Cadmium burdens and concentrations in transplanted chironomids initially decreased but, subsequently, Cd accumulated at a slower rate than controls. As a result of slower growth, however, Cd concentrations in transplanted larvae increased more rapidly than in controls. Aluminum content and concentration increased in control larvae more rapidly than in

transplanted individuals. Manganese, Ni, and Cu concentrations and burdens increased in transplanted larvae, while burdens increased but concentrations remained unchanged (Mn and Cu) or increased to a lesser extent (Ni) in control larvae. Less Zn was accumulated by transplanted than by control larvae. With the exception of an initial increase in Zn concentrations for transplanted chironomids, Zn concentrations remained constant for control and transplanted Plastic Lake larvae. Calcium concentrations in transplanted larvae decreased markedly within the first 3 d, but stayed fairly constant for the remainder of the experiment. Calcium content and concentration increased more rapidly in control than in transplanted Plastic Lake larvae. Increases in Fe concentration and content for transplanted larvae were more rapid in the initial than in the later phase of the experiment, and exceeded Fe accumulation rates in control larvae throughout the experiment.

Discussion

Surface Adsorption Versus Tissue Incorporation

For organisms of similar size and metabolic activity, differences in body burdens between populations should be restricted to physicochemical processes if physiological pathways for metal accumulation are the same for both populations. These processes could involve the reestablishment of new chemical equilibria between the cuticle and surrounding environment. Surface exchange processes should be rapid relative to absorption and retention processes, and initial changes in body burdens would probably be associated with adsorptive phenomena. Subsequent changes could be attributed, in part, to the physiological uptake or elimination of metals. In the initial 3 d of exposure, both populations that were held in spiked sediments increased their Cd, Ni, Pb, and Zn body burdens. From results of a concurrent study (Krantzberg and Stokes 1988) surface adsorption accounted for a greater proportion of Pb and Al body burdens in Plastic Lake than in L240W larvae. When Plastic Lake larvae were placed in L240W sediments, they initially lost Pb and Al. Conversely, L240W larvae gained Pb and Al when held in Plastic Lake sediments. These early changes can be attributed to surface adsorption and the reestablishment of chemical equilibria with sediments containing different pools of biologically reactive metals.

The initial rate of Cu accumulation was more rapid in Plastic Lake larvae than in L240W larvae held in spiked sediments. The sediment extraction procedure produced patterns of Cu partitioning for spiked sediments that approached those of L240W sediments (Fig. 2). This could account for the absence of a rapid initial phase of Cu accumulation when L240W larvae were held in spiked sediments. That is, if surface adsorption was important for the initial phase of Cu uptake, the epicuticle of L240W larvae could have been close to equilibrium with the spiked sediments by virtue of preexposure to L240W sediments.

The changes in metal burdens of L240W chironomids in control Plastic Lake sediments further illustrate the short-term phenomena that would be associated with adsorption. Control sediments had less total extractable Cd, Cu, and Ni than did L240W sediments. When placed in control sediments, L240W larvae decreased their Cd, Cu, and Ni contents. This may have been due to the displacement of those elements from surface binding sites by other cations that could compete more effectively for adsorption sites due to the lower concentrations of "available" Cd, Cu, and Ni in Plastic Lake as compared with L240W sediments. In a concurrent study, surface adsorption accounted for up to 75% of the total metal burden in chironomids collected from Plastic Lake and L240W (Krantzberg and Stokes 1988). Short-term fluctuations in metal burdens observed here, then, are likely to be a result of adsorptive phenomena.

Evidence for Metal Regulation and Tolerance

When Plastic Lake larvae were held in L240W sediments, within 8 wk their growth, as measured by changes in biomass, declined relative to all other larvae (Fig. 4). The effects of metal toxicity on growth in chironomids have been documented for acute and chronic exposures. Anderson et al. (1980) reported that at high aqueous Cu and Cd concentrations, growth in *Tanytarsus dissimilis* was reduced. Head capsule and nest tube widths, and reproductive success declined in *Paratanytarsus parthenogeneticus* with increasing Cu concentrations (Hatekeyama and Yasumo 1981). Total protein content of *Chironomus tentans* larvae decreased following exposure to elevated Cd and Pb concentrations (Rathore et al. 1979). Chronic exposure to elevated Ni concentrations substantially reduced larval growth (Powlesland and George 1986) and similar effects of Cd, Zn, and Cr on larval development were reported by Wentsel et al. (1977).

The reduced growth rates that were observed for transplanted Plastic Lake larvae indicate that these chironomids were physiologically impaired. The growth rates of L240W larvae were comparable with those of Plastic Lake controls and greater than those for Plastic Lake transplanted larvae (Fig. 4). L240W chironomids, then, did not appear to have been under physiological stress as compared with Plastic Lake transplanted larvae. The controls demonstrated that growth retardation was not simply due to stress induced by handling and it seems reasonable to conclude that the L240W population was more tolerant of contaminated sediments. The sensitivity of Plastic Lake fourth instar larvae to L240W sediments is significant because several studies have demonstrated that fourth instar larvae are substantially less sensitive to metal contamination than are earlier instar larvae (Anderson et al. 1980; Gauss et al. 1985; Williams et al. 1986; Powlesland and George 1986). Nevertheless, we were able to detect effects on growth rates in fourth instar larvae.

If L240W larvae are more tolerant to elevated metal levels in sediments, they may have the ability to detoxify and/or regulate the absorbed, or internal portion of their body burdens. While both populations accumulated Cu and Ni more rapidly in treated (spiked) compared with control sediments, the rates of accumulation for Plastic Lake larvae exceeded those for L240W larvae. The results for Cu were particularly striking, with Plastic Lake chironomids accumulating Cu 5 × more rapidly than L240W larvae. The degree of Cu and Ni accumulation by transplanted Plastic Lake larvae substantially exceeded that of larvae in their own sediments. Furthermore, L240W larvae were able to eliminate Cu and Ni when held in Plastic Lake sediments. It appears, then, that L240W larvae can indeed regulate Cu and Ni. This is similar to the results of Hopkin et al. (1985) who showed that while Cu concentrations were greater in isopods from contaminated sites than from less contaminated sites, they were not as high as one would predict based on the extent of Cu contamination. Copper tolerance has been associated with the ability to regulate internal Cu levels (Bryan et al. 1983; Amiard-Triquet et al. 1983). Hutchinson et al. (1975) showed that Ni was excluded by clams and crayfish collected

from contaminated sediments. This apparent ability to regulate Cu and Ni may have evolved as a response to the elevated content of these elements in L240W sediments (Fig. 2). While the causative nature of Cu and Ni regulation by L240W chironomids appears to be the elevated Cu and Ni concentrations historically present in L240W sediments, this explanation remains speculative. The differences between the two populations could also be due to genetic drift or founder effects, or could reflect differences in species or subspecies assemblages. Further examination of several populations would assist in validating that the differences in the capacity to regulate metals were, in fact, a consequence of different levels of sediment contamination.

Plastic Lake larvae in L240W sediments increased their biomass by 42% of L240W controls. Hypothetically, if Plastic Lake larvae accumulated metals from L240W sediments at a rate that was proportional to that of native larvae, after an initial period of surface adsorption or desorption, Plastic Lake larvae would increase their body burdens by 42% that of L240W larvae. Transplanted larvae accumulated 77% as much Mn as L240W controls, which is 33% more than predicted if both populations were metabolizing Mn in the same manner. Furthermore, L240W larvae were able to eliminate Mn when held in Plastic Lake sediments. This suggests that L240W chironomids can, to some extent, regulate Mn, as well as Cu and Ni.

L240W transplanted larvae accumulated Pb and Cd at the same rate as Plastic Lake controls, and Plastic Lake transplanted chironomids accumulated 40 and 44% as much Pb and Cd, respectively, as L240W controls. The extent of Pb and Cd accumulation was close to the expected value of 42%. It appears that chironomids do not regulate Pb or Cd. In addition, both of the chironomid populations exposed to treated (spiked) sediments accumulated Pb and Cd in the 3–14 d interval at rates that were similar to one another but greater than in unspiked (control) sediments, reflecting the greater bioavailability of these metals in spiked sediments, and demonstrating that they apparently lacked the ability to regulate Pb and Cd. The inability to regulate Pb and Cd in response to increasing environmental levels has been documented for mussels (Schulz-Baldes 1974; Davies and Pirie 1980; Satsmdjis and Voutsinou-Taliadouri 1983; Luten et al. 1986), snails (Bryan et al. 1983), oysters and clams (Byrne and DeLeon 1986), larval prawns (Devineau and Amiard-Triquet 1985), crustaceans (Evans and Lasenby 1982; Spehar et al. 1978), earthworms (Ireland 1975; Roberts and Johnson 1978), and insects and crayfish (Anderson et al. 1978). In contrast, all larvae maintained a constant Zn concentration, regardless of the sediment to which they were exposed. This apparent ability to regulate Zn agrees with the general ability of many invertebrates to regulate Zn (Kormondy 1965; Bryan et al. 1977; Hopkin and Martin 1984) and has frustrated many attempts to use an array of organisms as biological indicators of environmental Zn levels.

Iron metabolism is closely linked with hemoglobin synthesis in *Chironomus*. Tichy (1980) showed that hemoglobin content increases dramatically with growth. Jernelov et al. (1981) found that *C. riparius* collected from an acid pond in Canada has almost twice the relative hemoglobin concentration of the same species in Scandinavia. They speculated that this, coupled with the greater buffering capacity of the hemoglobin in the former population, conferred greater resistance to acidity.

Iron accumulation by L240W larvae in Plastic Lake sediments was comparable with Plastic Lake controls, except for the initial loss of Fe by L240W larvae. L240W larvae accumulated Fe more rapidly when in their own sediments than when in Plastic Lake sediments and Plastic Lake larvae also accumulated Fe more rapidly when in L240W sediments. The greater Fe uptake by both populations when held in L240W sediments may be a result of increased hemoglobin synthesis, although hemoglobin concentrations were not measured. If Fe accumulation was associated with hemoglobin synthesis, it is not surprising that Plastic Lake transplanted larvae had less Fe at the termination of the experiment than did L240W controls. The transplanted larvae did, however, have more Fe than one would predict based on biomass changes (60 versus 42%). This may also be related to the greater metal stress that Plastic Lake transplanted larvae were experiencing. Other studies have shown that hemoglobin concentrations can increase as a response to stress (Neville 1979; Fromm 1980).

One notable difference between the two populations was revealed by the accumulation patterns of Ca. While Ca accumulation did not differ significantly between L240W transplanted and Plastic Lake control larvae, Ca accumulation by Plastic Lake transplanted larvae was markedly depressed from the levels of Plastic Lake and L240W controls. In addition, L240W controls accumulated more Ca than did their transplanted counterparts despite the higher values for extractable Ca and CaE/FeE in Plastic Lake than in L240W sediments. It appears that L240W chironomids in contaminated sediments were more efficient at sequestering or retaining Ca than were Plastic Lake chironomids. This could confer greater resistance to elevated metals. Calcium–metal interactions and the amelioration of metal toxicity by Ca have been reported for a wide range of invertebrates (Ireland and Richards 1977; O'Shea and Mancy 1978; Coombs 1979; Brown 1982; Morgan 1986). The impaired Ca accumulation exhibited by Plastic Lake transplanted larvae may have contributed to their increased sensitivity to L240W sediments.

We suggest that following the initial phase of metal uptake that may have reflected surface adsorption processes and subsequent, slower rates of accumulation reflected uptake through metabolic pathways. This type of biphasic pattern was shown in a laboratory study on marine invertebrates (Ray et al. 1981), where Cd and Pb concentrations continued to increase with time while Cu and Zn were regulated. In summary, Cd and Pb were not regulated, Cu and Ni were regulated to some extent by chironomids collected from the contaminated L240W sediments, and Zn was regulated by both populations.

Ecological Implications

Exposure to contaminated sediments caused growth retardation in chironomid larvae collected from a less contaminated environment. This has implications for episodic or pulse metal loadings. Chironomid larvae are important fish prey items (Berrie 1972). Brown et al. (1980) reported that chironomid larvae and pupae contributed 20% by weight to the total diet of brown (*Salmo trutta*) and rainbow trout (*Salmo gairdneri*). Emergent chironomids can account for up to 60% of the diet of many avian species at certain times of the year (Titmus and Badcock 1980). Wentsel et al. (1978) reported delayed and depressed chironomid emergence when larvae were exposed to metal-contaminated sediments. Metal contamination, then, could result in a substantial reduction in food quantity and/or quality for aquatic and terrestrial wildlife. We have shown that growth in chironomids decreased and metal accumulation increased when larvae from a less polluted site were exposed to contaminated

sediments. With chronic exposure, tolerant populations may develop through time. However, pulse metal loadings could substantially impact chironomids and subsequently, their predators.

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Effect of Cadmium on a Stable, Large Volume, Laboratory Ecosystem Containing *Daphnia* and Phytoplankton

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Borgmann, U., E. S. Millard, and C. C. Charlton. 1989. Effect of cadmium on a stable, large volume, laboratory ecosystem containing *Daphnia* and phytoplankton. Can. J. Fish. Aquat. Sci. 46: 399–405.

Stable 3400-L indoor *Daphnia*-phytoplankton ecosystems were exposed to cadmium to test the hypothesis that sublethal concentrations of toxicants cause an increase in the biomass of lower trophic levels and a decrease in the biomass of higher trophic levels. At 5 $\mu g \cdot L^{-1}$ the *Daphnia* population collapsed after approximately 9 wk and chlorophyll levels increased to levels substantially greater than those observed in the control. At 15 $\mu g \cdot L^{-1}$ the *Daphnia* population declined after approximately 5 wk. Chlorophyll levels remained low even after the *Daphnia* collapse. At concentrations of 1 $\mu g \cdot L^{-1}$ and below, the ecosystem appeared to remain stable. The nature of the response of the ecosystem to 5 $\mu g\ Cd \cdot L^{-1}$ was predicted correctly, but the magnitude of the response was not. A synergistic effect between cadmium toxicity and *Daphnia* population growth inhibition by high algal concentrations resulted in a much more dramatic response than predicted. A standard benchtop *Daphnia* bioassay could be used to accurately predict the concentration of cadmium below which no detrimental ecosystem effects are likely to occur; however, the degree of response cannot be predicted.

Des écosystèmes intérieurs stables (3400 L) de *Daphnia*-phytoplancton ont été exposés au cadmium afin de vérifier l'hypothèse selon laquelle des concentrations sublétales d'une substance toxique peut provoquer une augmentation de la biomasse dans les niveaux trophiques inférieurs et une diminution de la biomasse dans les niveaux trophiques supérieurs. A 5 $\mu g \cdot L^{-1}$, la population de *Daphnia* s'est effondrée au bout d'environ 9 sem et la teneur en chlorophylle a augmenté jusqu'à des niveaux considérablement supérieurs à ceux observés dans les écosystèmes témoins. A 15 $\mu g \cdot L^{-1}$, la population de *Daphnia* a régressé au bout d'environ 5 sem. La teneur en chlorophylle est restée faible même après l'effondrement des populations. A la concentration de 1 $\mu g \cdot L^{-1}$ et moins, l'écosystème a paru rester stable. La réponse de l'écosystème à l'exposition à 5 $\mu g \cdot Cd \cdot L^{-1}$ a été prévue avec justesse, mais pas son ordre de grandeur. Il y eu synergie entre la toxicité du cadmium et l'inhibition de la croissance de *Daphnia* à des concentrations algaires élevées qui a considérablement accentué la réponse attendue. Donc, il serait possible d'utiliser un essai biologique uniformisé en laboratoire avec *Daphnia* pour prédire avec justesse la concentration de cadmium en deçà de laquelle aucun effet toxique pour l'écosystème n'est à prévoir en toute probabilité. Cependant, l'intensité de la réponse n'est pas prévisible.

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Most toxicity bioassays concern the effect of toxicants on single species in isolation. How these same organisms would respond to the toxicant in the field, where other species and trophic interactions also occur, is difficult to predict from laboratory tests alone. Recent developments in ecosystem modelling have made it possible to predict biomass and production over different trophic levels in the pelagic ecosystems of lakes by classifying organisms according to body size (Borgmann et al. 1984; Borgmann 1987; Leach et al. 1987). Borgmann (1985) adapted this approach to demonstrate how the ecosystem response to a toxic substance might be predicted from laboratory dervied toxicity data. It was predicted that substances which exert their toxicity by reducing biomass conversion efficiencies would reduce the biomass at higher trophic levels and increase the relative amount of biomass at the lower trophic levels. This is a testable hypothesis if experiments at the ecosystem scale can be performed.

Ecosystem level experiments with toxic substances have been attempted with both metals and organic contaminants (e.g. Hurlbert et al. 1972; Menzel and Case 1977; Gächter 1979; Marshall and Mellinger 1980; Kettle and deNoyelles 1986; Day et al. 1987; Kerrison et al. 1988), but such experiments have usually not been conducted under strictly controlled conditions where the stability and steady-state population levels of the test organisms have been established prior to addition of the toxicant. However, Borgmann et al. (1988) demonstrated that phytoplankton and *Daphnia* can coexist in a stable ecosystem for up to 20 wk in large volume (3400 L) indoor mesocosms. Stability was demonstrated by damped oscillations in algal and *Daphnia* biomasses and by a return to steady-state conditions if the system is disturbed. Algal and *Daphnia* biomasses responded to different nutrient loading and *Daphnia* harvesting rates in a predictable fashion. Such an experimental ecosystem provides an opportunity to determine ecosystem response to a toxicant and to test the hypothesis that lower trophic level biomass should increase relative to the biomass at higher trophic levels under sublethal toxic stress.

We describe here the effect of cadmium on the *Daphnia*-phytoplankton experimental ecosystem. Cadmium was chosen as a representative toxicant because it has been well studied in

laboratory assays (Biesinger and Christensen 1972; van Leeuwen et al. 1985; Elnabarawy et al. 1986; Knowles and McKee 1987), and because it exerts chronic toxicity by decreasing biomass conversion efficiency in fish (Borgmann and Ralph 1986). Our a priori prediction of the ecosystem response was that the long term average biomass of the lower trophic level (algae) should increase relative to the biomass of the upper trophic level (zooplankton) at sublethal cadmium concentrations (Borgmann 1985), assuming cadmium concentrations are not high enough to inhibit algal growth completely. This is expected because the decreased biomass conversion efficiency of the zooplankton means that a larger biomass of phytoplankton is required to support a given biomass of zooplankton. A secondary objective of this study was to test the sensitivity of standard benchtop bioassays in predicting the toxicant concentration likely to affect the ecosystem.

Methods

Ecosystem experiments were carried out in four stainless steel columns 4.5 m high and 1.0 m in diameter. The methods used in establishing and sampling the experimental ecosystems are described in detail in Borgmann et al. (1988) and one of the columns is illustrated in Millard et al. (1983). The columns were filled with Burlington city tap water originating from Lake Ontario. This has a hardness of about 130 $mg \cdot L^{-1}$, an alkalinity of 90 $mg \cdot L^{-1}$, and a cadmium concentration of <0.3 $\mu g \cdot L^{-1}$. The pH, which was not deliberately controlled during the experiments, ranged from 8.2 to 8.6 (Lake Ontario ranges from 7.7 to 8.3, Johannsson et al. 1985). The columns were mixed vertically by pumping surface water with a magnetic drive pump through a stainless steel tube down the inside of the column with the outlet at 3.5 m and directed toward the surface. A 400-μm nylon mesh screen covering the pump intake prevented *Daphnia* from being sucked through the pump. The columns were isothermal from top to bottom, the temperature varying between 19 and 22°C, depending on the ambient air temperature. Irradiance to each column was provided by a 1 kW metal-halide lamp with aluminum parabolic reflector (Canadian General Electic Co.). Light intensity (400 – 700 nm) was between 750–1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the water surface in the centre of each column.

Inocula of algae were from batch cultures of *Chlorella* sp. grown in Bristol's medium but deficient in phosphorus relative to other nutrients. The dominant algal species in column 1 were *Phacotus* sp. and *Scenedesmus quadricauda* during the first chlorophyll peak. *Chlorella* sp. was the dominant algae for the remainder of the experiment, with some *Oochromonas* appearing near the end. Phosphorus was maintained as the limiting nutrient (loading rate 0.5 $mg \cdot column^{-1} \cdot d^{-1}$). All other essential macro- (C, N, Mg, Si, Fe) and micro-nutrients (Cu, Co, Mn, Mo, Zn) were added in excess. Nutrients were added daily from Monday to Friday, with a single dose on Tuesday to Thursday and a double dose on Friday and Monday. Composite samples of chlorophyll *a* were collected twice a week by pooling water drawn from three depths by peristaltic pump. These were filtered through GF/C filters (Whatman Co.) and analyzed according to Burnison (1980) using dimethyl sulfoxide as the extractant.

Inocula of zooplankton were from laboratory cultures of *Daphnia magna* maintained in our laboratory for several years and raised on *Chlorella*. *Daphnia* harvesting and sampling for other parameters began several weeks after addition of the *Daphnia*, when visual inspection of the columns suggested that a sizable *Daphnia* population had developed. *Daphnia* were harvested twice a week using a 65-cm diameter, 400-μm mesh plankton net hauled vertically up the center of the column from a depth of 1.3 m off the bottom (the length of the net from collar to end of collection bucket) to the surface. The columns were mixed for 5 – 10 min before sampling. This resulted in removal of approximately 16% of the *Daphnia* on each sampling date and is equivalent to an instantaneous harvesting rate of $0.051 \cdot d^{-1}$ (Borgmann et al. 1988). *Daphnia* biomass was determined by rinsing an aliquot of the harvested *Daphnia* in distilled water, drying at 60°C in preweighed aluminum foil pans, ashing, and reweighing. Another subsample was preserved in sugared formalin for determination of lengths and egg abundance.

Cadmium was added as $CdSO_4$, and was replenished as required when concentrations fell below the desired level. Cadmium concentrations of 1 and 5 $\mu g \cdot L^{-1}$ were chosen initially. We guessed that one of these might be chronically toxic to our *Daphnia*, based on published studies. Six weeks after sampling began the 1 $\mu g \cdot L^{-1}$ concentration was added to C3. This was increased to 15 $\mu g \cdot L^{-1}$ 5 wk later (week 11), when it appeared that neither the 1 nor the 5 $\mu g \cdot L^{-1}$ treatments might be toxic. The 5 $\mu g \cdot L^{-1}$ concentration was added to C2 at week 5. Cadmium in water was measured twice weekly with a Hitachi 180-80 graphite-furnace, atomic absorption spectrophotometer by K. Lum, National Water Research Institute, Department of Environment, Burlington. Cadmium loss from the water column (% per day) was calculated from the difference between initial (after the first spike) and final concentrations, plus the sum of all cadmium additions, divided by the number of days at a given treatment and the mean water concentration. Cadmium loss due to harvesting of the *Daphnia* was ignored, since this amounted to less than 0.1% per day of the cadmium present in the water column. Cadmium levels in individual *Daphnia* from two of the columns were provided by N. Yan, Ontario Ministry of the Environment, Dorset. Cadmium concentrations in bulk samples of *Daphnia* were determined by atomic absorption spectrophotometry by the Water Quality National Laboratory, Inland Waters Directorate, Department of Environment.

Benchtop chronic cadmium toxicity experiments were performed using procedures similar to Biesinger and Christensen (1972). The test was initiated with 0 – 24 h old *Daphnia* placed individually in 200 mL of water in 250-mL polypropylene bottles. Animals were incubated at 20°C with a photoperiod of 12 h light and 12 h dark and an average pH of 8.5. Ten replicates were used at each cadmium concentration, but those which turned out to be males (0 – 3 out of 10 individuals at each concentration) were disregarded when calculating mean reproductive rates. The animals were fed washed *Chlorella* sp. at a concentration of 1 $mg \cdot L^{-1}$ (dry weight) during the first week, and 2 $mg \cdot L^{-1}$ during the rest of the experiment. Preliminary experiments indicated that this provided sufficient food to give near maximum reproductive rates. The water, food, and cadmium were changed twice a week, at which time the young were counted and removed. Cadmium concentrations of 0, 2, 5, 10, 15, and 25 $\mu g \cdot L^{-1}$ were chosen to include the concentrations used in the model ecosystems plus concentrations bracketing these in a roughly logarithmic fashion. Cadmium concentrations in the water after each incubation period were measured by the Water Quality National Laboratory.

The instantaneous rate of population increase (r) in the benchtop experiments was calculated from

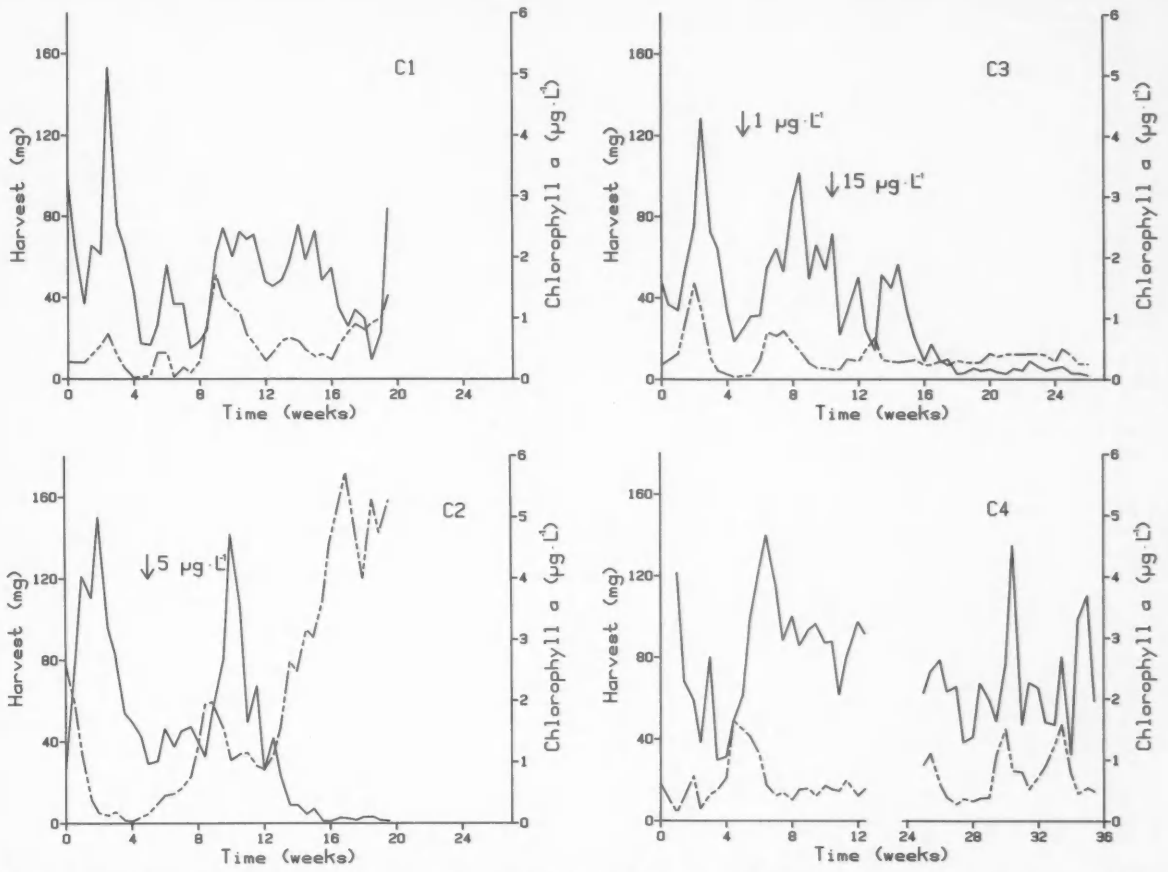

FIG. 1. *Daphnia* harvested (milligrams ash free dry weight, solid line) and chlorophyll *a* (broken line) as a function of time for each of the four experimental water columns. Arrows indicate time of cadmium addition and target cadmium concentration. The harvesting rate was not maintained constant in column 4 during the time interval missing in the figure.

$$1 = \Sigma e^{-rti} Y_i,$$

where t is the time between water changes (3.5 d) and Y_i is the total number of young produced during the ith time interval divided by the initial number of females (equal to fraction of females surviving × young produced per surviving female, e.g. Michod and Anderson 1980). The summation was calculated over the entire 10-wk experimental period, but r values calculated using only the first 3 wk data are almost identical. This equation does not give an exact estimate of r. In effect, it gives what r would be if all the young were produced at the end of each time interval. This is, therefore, a lower limit for r. The upper limit for r can be calculated by assuming all the young were produced at the beginning of each time interval, or at the end of the previous time interval. The upper limit of r was therefore calculated using the above equation, but replacing Y_i with Y_{i+1}.

Benchtop tests were also performed to determine the growth rate of young (0 – 24 h old) *Daphnia* in various concentrations of *Chlorella* sp. as food. Twenty young daphnids were placed in 2 L of water in straight-sided pyrex glass jars to which were added various concentrations of the same culture of *Chlorella* used to inoculate the experimental ecosystems. The algal culture was first centrifuged and rinsed. The density of the resuspended algae was then estimated from turbidity measurements and appropriate dilutions were made. Aliquots of the test solution with algae were filtered through glass fibre filters, dried, and weighed, at both the beginning and the end of the 3-d incubation. The solutions were stirred several times each day. Initial and final algal density in each jar were averaged to obtain a mean measured phytoplankton concentration. Growth rates of

TABLE 1. Mean and standard deviations (SD) of cadmium in the water columns ($\mu g \cdot L^{-1}$) and in the *Daphnia* ($\mu g \cdot g^{-1}$ dry weight).

		Measured Cd		Cd in *Daphnia*	
Column	Nominal	Mean(*n*)	SD	Mean(*n*)	SD
C1	0	0.21(33)	0.05	21.9(3)	3.8
C2	0	0.08(11)	0.02	13.2(13*)	5.6
	5	4.71(23)	0.71	86.6(1)	—
C3	0	0.13(6)	0.01	—	—
	1	0.98(10)	0.11	38.9(10*)	14.9
	15	12.00(28)	2.53	157.0(1)	—
C4(a)	0	0.61(21)	0.08	25.3(2)	11.3
(b)	0	1.05(5)	0.16		

*Based on single *Daphnia*; the rest are bulk samples.

TABLE 2. Cadmium loss rates (percent per day) at various cadmium concentrations in the water ($\mu g \cdot L^{-1}$), either before or after the collapse of the *Daphnia* population.

Column	*Daphnia* collapse	Nominal	Measured	Loss rate
C2	no	5	4.54	1.63
	yes	5	5.27	0.10
C3	no	1	0.98	1.36
	no	15	13.17	1.56
	yes	15	10.62	4.99

TABLE 3. Eggs carried per female *Daphnia* of various body lengths (mm) averaged over several weeks. Sample size is 81–242 *Daphnia* per size–class up to 2.9 mm, and 30 – 78 for the 3.0 – 3.1 mm size–class. Data for C3 include samples collected either before or after addition of 15 μg $Cd \cdot L^{-1}$. * Values with the same superscripts have overlapping 95% confidence limits within each size class.

	Cadmium concentration				
	15	≤1			5
Length	C3	C3	C1	C4	C2
2.4–2.5	0.84[a]	0.60[a]	1.25	1.77[b]	1.83[b]
2.6–2.7	0.96[a]	1.04[a]	1.87[b]	2.18[b]	2.86
2.8–2.9	1.33	2.74[a]	2.20[a]	2.58[a]	2.64[a]
3.0–3.1	0.82	2.06[a]	2.08[a]	3.05[a]	2.53[a]

the *Daphnia* were calculated from the dry weight of the surviving animals and the initial dry weight estimated by weighing a subsample of young daphnia at the beginning of the incubation. We assumed the weight increase was exponential during the 3-d test.

Results

Cadmium concentrations below 1 $\mu g \cdot L^{-1}$ did not appear to seriously affect *Daphnia* or chlorophyll abundance in the ecosystem experiments (Fig. 1). Cadmium in the control (C1), and in the treated columns before cadmium addition (C2 and C3) was at or below 0.2 $\mu g \cdot L^{-1}$. One of the columns (C4) must have received metal contamination from some source, perhaps a welding joint, since cadmium levels were 0.6 $\mu g \cdot L^{-1}$ initially, and increased to 1 $\mu g \cdot L^{-1}$ by the end of the experiment (Table 1). The *Daphnia* harvest and chlorophyll concentrations in this column, however, and in C3 after addition of 1 $\mu g \cdot L^{-1}$, did not differ significantly from the control. We left C3 at 1 $\mu g \cdot L^{-1}$ for only about 5 wk because both C3 and C2 (now at 5 $\mu g \cdot L^{-1}$) showed no response to cadmium by this time. In hindsight, this time period is insufficient to observe a response at low cadmium concentrations. The plankton in C4, however, were exposed to a similar concentration (0.61–1.05 $\mu g \cdot L^{-1}$) for much longer. Exposure to 0.6 $\mu g \cdot L^{-1}$ elicited no response after about 13 wk. The harvesting rate was not maintained constant for the next 12 wk, so these data cannot be compared with the rest of this study. After 25 wk, harvesting was again held constant for an additional 11 wk. Cadmium levels had risen to about 1 $\mu g \cdot L^{-1}$ by this time, but the *Daphnia* harvest and chlorophyll concentrations still appeared to be stable (Fig. 1).

Both the 5 and 15 μg $Cd \cdot L^{-1}$ additions had marked, but different, effects on the ecosystems. The *Daphnia* in column 2 decreased dramatically after approximately 9 wk exposure to 5 $\mu g \cdot L^{-1}$, and never recovered. Chlorophyll levels increased after the *Daphnia* crash to the highest level seen in this experiment (5 $\mu g \cdot L^{-1}$, Fig. 1), and the column remained visually very green. Addition of 15 $\mu g \cdot L^{-1}$ resulted in a marked drop in *Daphnia* abundance after about 5 wk (Fig. 1, C3). Chlorophyll levels remained low, however. Visually, the water in C3 became very transparent, even before the *Daphnia* crash, and the bottom could be seen clearly. This is in sharp contrast to the response observed in C2 with 5 μg $Cd \cdot L^{-1}$, and demonstrates that cadmium is directly toxic to algae at this concentration.

During exposure to the 1, 5, and 15 $\mu g \cdot L^{-1}$ levels, cadmium had to be added in varying amounts (except for C4) to replace that which was apparently sedimenting as algae, faecal pellets, or detritus. As long as the *Daphnia* harvest was similar to control values, the cadmium loss from the water column was approximately 1.5% per day, regardless of the total cadmium concentration (Table 2). After the *Daphnia* crash, the cadmium level in the 5 $\mu g \cdot L^{-1}$ treatment remained virtually constant without further cadmium additions. In contrast, the cadmium loss rate at 15 $\mu g \cdot L^{-1}$ increased to about 5% per day after the *Daphnia* had declined (Table 2).

The concentration of cadmium in the *Daphnia* increased with increasing water concentrations. A double logarithmic regression of body concentrations versus water levels (Table 1) gave a slope of 0.49 and an R^2 of 0.96, indicating that body levels were approximately proportional to the square root of water levels.

The numbers of eggs carried per adult *Daphnia* were determined for different sized females to see if egg frequency might be a useful indicator of toxic stress. *Daphnia* less than 2 mm long did not carry eggs. There was some variability in eggs per female among columns 1, 3, and 4 for small animals (up to 2.7 mm) at ≤1 μg $Cd \cdot L^{-1}$, but the number of eggs per female were similar for 2.8 – 3.1 mm animals (Table 3). At 5 $\mu g \cdot L^{-1}$ the *Daphnia* did not show any reduction in the number of eggs carried per female. In fact, there appears to be a modest increase in the number of eggs in the smaller animals. At 15 $\mu g \cdot L^{-1}$, the smaller animals carried fewer eggs than two of the columns (C1 and C4) at low cadmium levels. The larger animals carried significantly fewer eggs than all other treatments. The animals exposed to 1 $\mu g \cdot L^{-1}$ or less showed a clear increase in number of eggs carried with increasing body size. This was much less evident in the 15 $\mu g \cdot L^{-1}$ treatment (Table 3).

To check for adaptation to cadmium we attempted to determine 48-h LC50 values for 0 – 24 h old young obtained from females collected from the experimental columns. Sufficient young were not obtained to accurately estimate the LC50, but some adaptation did appear to occur. At 560 $\mu g \cdot L^{-1}$ all *Daphnia* (59/59) obtained from C1 died within 48 h. However, 18% (12/68) from C4 survived (significantly different from C1 at $P < 0.01$, chi-square), including at least one from each of the seven separate tests done (8 – 10 *Daphnia* per test), and 24% (4/17) from C3 survived (using young obtained after addition of 15 μg $Cd \cdot L^{-1}$; significantly different from C1 at $P < 0.01$). At 320 $\mu g \cdot L^{-1}$, survival was 41% (32/78), 44% (33/75), and 69% (11/16) for animals obtained from C1, C4, and C3, respectively. Adaptation to cadmium did, therefore, occur in this experiment, even after exposure to concentrations of only 1 $\mu g \cdot L^{-1}$ (C4), but this resulted in only a small increase in tolerance to cadmium.

Cadmium toxicity in the benchtop chronic *Daphnia* bioassay was similar to that observed in our mesocosm experiments. The LC50 lay between 13 and 23 $\mu g \cdot L^{-1}$ after 3 wk, but dropped

TABLE 4. Survival, young produced per female *Daphnia*, and instantaneous population growth rate (r) at various cadmium concentrations ($\mu g \cdot L^{-1}$) in the chronic bioassay. Both lower and upper estimates of r are given, assuming the young were produced at either the end or the beginning of each time interval (see text).

Cadmium concentration		% Survival		Young/female		r
Nominal	Measured (SD)	3 wk	8 wk	3 wk	8 wk	(d^{-1})
0	0.22 (0.12)	100	86	67	157	0.29–0.40
2	1.86 (0.25)	100	67	54*	118	0.27–0.37
5	4.10 (1.01)	100	0	36*	58*	0.26–0.35
10	7.78 (1.92)	67	0	12*	12*	0.18–0.23
15	13.43 (0.79)	67	0	10*	10*	0.16–0.22
25	22.92 (2.22)	0	0	11*	11*	0.17–0.23

*Significantly different from control at $P < 0.05$.

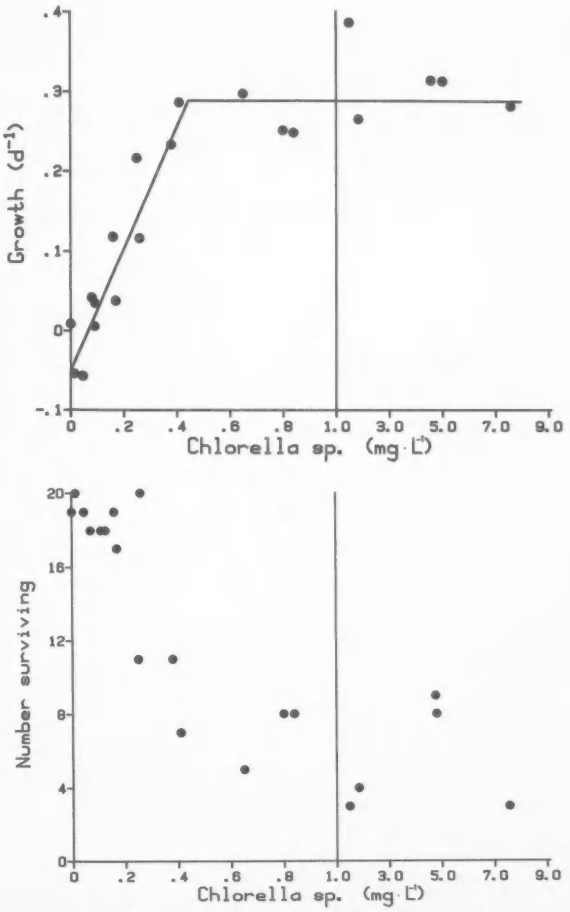

FIG. 2. Growth rate (top) and number surviving of 0–24 h old *Daphnia* during 3-d exposures to various concentrations of *Chlorella* sp. Note the 10-fold scale change for the x axis on the right side of the figure. The diagonal line is the linear regression for all growth measurements at <0.5 mg *Chlorella*·L^{-1}. The horizontal line is the mean growth rate at >0.5 mg *Chlorella*·L^{-1}.

to between 2 and 4 $\mu g \cdot L^{-1}$ after 8 wk (Table 4). A significant reduction in the number of young produced per female was observed at 2 $\mu g \cdot L^{-1}$ after 3 wk. The instantaneous rate of population increase (r) calculated from these data dropped slightly at 4 $\mu g \cdot L^{-1}$, and more dramatically at 8 $\mu g \cdot L^{-1}$. The toxicity of cadmium in the chronic bioassay was, therefore, very similar to the toxicity based on the mesocosm response.

The growth rate of young *Daphnia* increased with increasing food concentration, and then levelled off in an almost rectilinear fashion (Fig. 2). The break in the feeding curve occurred at approximately 0.45 mg *Chlorella*·L^{-1}. This concentration also roughly coincided with the food concentration above which the survival was consistently lower. At 0.25 – 0.26 mg·L^{-1} survival was variable, with 11 surviving in one test and all 20 in another. Mortality was higher at 1 mg *Chlorella*·L^{-1} in this experiment than in the chronic toxicity bioassays, where mortality of young *Daphnia* was low during the first week. This was probably due to the greater care we took to keep the algae suspended in the feeding experiment.

Discussion

The scale of our experiments, both in time and in physical size, prevented us from performing replicates of each treatment. We do, however have data for four separate runs under control or 1 μg Cd·L^{-1} conditions within this experiment: column 1 (which provided 18.5 wk of data under "steady-state" conditions), column 3 prior to addition of 15 μg Cd·L^{-1} (9 wk of data), and two separate times for column 4 (11.5 and 9 wk). We also have two runs from previous experiments (7 and 4 wk of data) under the same conditions. In all six cases the ecosystems appeared to be stable. Mean chlorophyll and *Daphnia* harvest for each run ranged from 0.38 to 0.77 $\mu g \cdot L^{-1}$ and 50 to 83 mg·column^{-1}·sampling day^{-1}, respectively (Borgmann et al. 1988). Furthermore, five additional runs at different harvesting and nutrient addition rates gave phytoplankton and zooplankton concentrations consistent with theoretical predictions. *Daphnia* populations, once established, never collapsed in any of these runs unless the harvesting rate was increased to eight net hauls per week, which appears to roughly equal the maximum growth rate of the *Daphnia* (Borgmann et al. 1988). This strongly suggests that the *Daphnia* population collapses seen after addition of 5 or 15 μg Cd·L^{-1} in the results reported here are, in fact, due to the presence of cadmium.

Our a priori prediction of the ecosystem response to cadmium at sublethal concentrations, that algal biomass should increase relative to the biomass of *Daphnia*, was confirmed (Fig. 1, C2). This prediction was based on the assumption that biomass conversion efficiency is reduced by cadmium in *Daphnia* as it is in fish (Borgmann and Ralph 1986). The shift in relative bio-

mass of *Daphnia* and phytoplankton would be independent of any direct toxicity of cadmium on algae (Borgmann 1985). A reduction in conversion efficiency of *Daphnia* would result in lower production rates for *Daphnia*, leading to a lower *Daphnia* biomass and reduced grazing pressure on algae. This allows the phytoplankton biomass to increase. The system should stabilize when the phytoplankton biomass has increased sufficiently to support the now reduced biomass of *Daphnia*.

Although the nature of the ecosystem response was correctly predicted at 5 $\mu g \cdot L^{-1}$, its magnitude was greater than expected. There was a virtual elimination of the *Daphnia* and a subsequent dramatic increase in chlorophyll (Fig. 1). The *Daphnia* collapse is probably the result of the synergistic effect of cadmium toxicity and a reduction in *Daphnia* population growth rates due to the higher algal densities. At the nutrient addition and harvesting rates used in this experiment, and in the absence of added cadmium, the *Daphnia* and phytoplankton always coexisted in a stable ecosystem, provided initial chlorophyll levels were not excessive. In one of our previous experiments, however, the phytoplankton biomass became quite high (chlorophyll >20 $\mu g \cdot L^{-1}$) before the *Daphnia* population could be established. We were unsuccessful in establishing a sizable *Daphnia* population in this column until chlorophyll levels dropped below 20 $\mu g \cdot L^{-1}$ after we kept the lights off for 6 wk. Once the *Daphnia* population was established and the lights turned on again, the system appeared to be stable. In 1-L benchtop assays the growth of young *Daphnia* increased with increasing additions of *Chlorella* sp. up to about 0.5 $mg \cdot L^{-1}$ dry weight, or roughly 1 $\mu g \cdot L^{-1}$ chlorophyll. At higher concentrations growth was constant. Mortality, however, was high at food concentrations of 0.4 $mg \cdot L^{-1}$ or greater (Fig. 2). Porter et al. (1983) also obtained an increase in mortality of *Daphnia* at higher algal concentrations, especially for newborn *Daphnia*, using *Chlamydomonas reinhardii* as food. Williamson et al. (1985) observed a similar increase in both growth and mortality of copepod nauplii with increasing algal concentrations. It is probable, therefore, that cadmium toxicity first caused a reduction in *Daphnia* biomass conversion efficiency at 5 $\mu g \cdot L^{-1}$. This resulted in a reduced *Daphnia* biomass, reduced grazing pressure and increased algal biomass. The increase in phytoplankton biomass appears to eventually have had a negative effect on *Daphnia* population growth. The *Daphnia* biomass would have decreased further, again reducing the grazing pressure. This allowed algal biomass to increase still further, creating a positive feedback loop which resulted in virtual elimination of *Daphnia* and the establishment of a high algal density. Such a condition, once established, cannot be reversed unless the algal biomass is drastically reduced by some other means (such as reduced light intensity).

If our experiment had been conducted in the field, where other zooplankton species are present, we might have found that the elimination of the *Daphnia* would have led to the establishment of other zooplankton populations, species more tolerant of cadmium and high algal densities. Marshall and Mellinger (1980) observed an increase in the abundance of several rotifer species as crustacean zooplankton declined at 1 μg $Cd \cdot L^{-1}$ in enclosures in the field. Yan and Geiling (1985) attributed the relative increase in rotifer abundance in acid-stressed lakes to decreased competition with crustacean zooplankton. Rotifer or smaller crustacean zooplankton might, therefore, be able to survive in our 5 $\mu g \cdot L^{-1}$ treatment, if introduced. In this respect, the presence of cadmium would have a similar effect as excessive fish predation. Heavy predation on large bodied zooplankton generally increases phytoplankton and small bodied zooplankton biomass, conditions regarded as characteristic of more eutrophic waters (e.g. Siegfried 1987). Pesticides have been observed to alter zooplankton and phytoplankton compositions in a similar fashion, leading to smaller zooplankton species (e.g. rotifers) and a higher phytoplankton biomass (e.g. Hurlbert et al. 1972). This makes it extremely difficult to distinguish between the effects of toxic substances, eutrophication, and overfishing. All three factors can cause similar ecosystem responses, and they probably often act in concert during anthropogenic environmental degradation.

In Lake Michigan, decreased planktivorous fish biomasses in recent years have been implicated in an increase in the abundance of larger *Daphnia* species, and subsequent decreases in algal biomass and increases in water clarity (Scavia et al. 1986). This is one of the goals of water quality management and can be achieved through biological manipulations. Planktivorous fish biomass is sometimes deliberately reduced in order to improve water clarity (e.g. Shapiro and Wright 1984). If other species of large *Daphnia*, such as *D. pulex*, *D. pulicaria*, and *D. galeata*, are affected by toxicants and algal concentrations in a similar way to *D. magna*, then the presence of a toxicant such as 5 μg $Cd \cdot L^{-1}$ could reverse this trend, negating attempts at water quality improvement.

Not only could an increase in cadmium reduce water clarity and *Daphnia* biomass, but it could also reduce the rate of cadmium sedimentation, possibly reducing the potential for future ecosystem recovery. As long as the cadmium concentration remains sufficiently low to allow the existence of *Daphnia*, the cadmium is cleared from the water column at a rate of about 1.5% per day (Table 2). Once cadmium toxicity eliminates *Daphnia*, and if cadmium concentrations are not high enough to also eliminate the phytoplankton, then the metal remains in the water column much longer. The *Daphnia*, therefore, appear to play an important role in the residence time of cadmium in the column. The sedimentation rate of other metals is also believed to be largely related to the sinking of zooplankton fecal material and exoskeletons (e.g. Fowler 1977).

We would have obtained completely different results if our experiments had been conducted for a shorter duration. The only quick response observed was that addition of 15 μg $Cd \cdot L^{-1}$ seemed to be directly toxic to the phytoplankton. The water column became visually quite clear shortly after this cadmium addition, and remained so even after the *Daphnia* crash. However, it took about 5 wk for the *Daphnia* harvest to drop substantially (Fig. 1). Had our tests lasted for only up to 5 wk, we would have concluded that 5 $\mu g \cdot L^{-1}$ was not toxic, and that 15 $\mu g \cdot L^{-1}$ reduces algal biomass but does not seriously affect *Daphnia*. Our final conclusion, however, is quite different. At constant *Daphnia* harvesting rates, the first expected effect of gradually increasing cadmium levels should be a reduction in *Daphnia* and an increase in phytoplankton due to decreased *Daphnia* conversion efficiency. This conclusion is only possible because our experiments lasted in excess of 10 wk after cadmium addition (Fig. 1).

The lowest cadmium concentration (between 1 and 5 $\mu g \cdot L^{-1}$) having a major impact on our mesocosms is similar to the lowest concentration having a significant effect in benchtop experiments (2 – 4 $\mu g \cdot L^{-1}$, Table 4). This implies that standard laboratory procedures may be reasonably accurate in predicting concentrations of toxicants which might seriously impact the same species in ecosystems. Cadmium toxicity to *D. magna* in our benchtop bioassay was similar to that reported for the same

species by others (e.g. van Leeuwen et al. 1985; Elnabarawy et al. 1986; Knowles and McKee 1987). Biesinger and Christensen (1972) observed a higher toxicity (3-wk LC50 = 5 $\mu g \cdot L^{-1}$, 50% reproductive impairment at 3 wk = 0.7 $\mu g \cdot L^{-1}$), but their study was conducted in softer water (hardness 45 $mg \cdot L^{-1}$). Field enclosure and pond experiments have also shown cadmium induced population changes in several daphnid and other cladoceran species at concentrations of 1 – 5 $\mu g \cdot L^{-1}$ (e.g. Marshall and Mellinger 1980; Kettle and deNoyelles 1986; Lawrence and Holoka 1987), in agreement with our ecosystem response.

One factor which could have resulted in a discrepancy between the benchtop and mesocosm test sensitivity to cadmium is adaptation, either physiological or through genetic selection. *Daphnia* adapt to metals to some degree (LeBlanc 1982). The animals used in our benchtop experiments were not adapted, but the mesocosm animals had several generations to adapt before the populations crashed. Mortality of young *Daphnia* obtained from the harvested animals and exposed to 320 or 560 $\mu g\ Cd \cdot L^{-1}$ demonstrated only a moderate degree of adaptation, however. This was apparently not sufficient to alter the long-term sensitivity of the mesocosm experiments, although preexposure to 1 $\mu g\ Cd \cdot L^{-1}$ in C3 may have delayed the *Daphnia* population decline after the cadmium concentration was increased to 15 $\mu g \cdot L^{-1}$. Benchtop experiments would appear, therefore, to be a valid method of estimating the minimum toxicant concentration detrimental to *Daphnia* in ecosystems, although the exact nature of the ecosystem response (e.g. the complete population crash in C2, Fig. 1) cannot be predicted. The number of eggs carried per female of a given length, on the other hand, was not a sensitive indicator of stress. This was observed to drop only at the highest cadmium concentration (Table 3), and is therefore unlikely to provide an early indication of toxic stress in natural populations.

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Genetic Divergence of Anadromous and Nonanadromous Atlantic Salmon (*Salmo salar*) in the River Namsen, Norway

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Vuorinen, J., and O. K. Berg. 1989. Genetic divergence of anadromous and nonanadromous Atlantic salmon (*Salmo salar*) in the River Namsen, Norway. Can. J. Fish. Aquat. Sci. 46: 406–409.

Anadromous and nonanadromous Atlantic salmon, *Salmo salar*, coexist in the River Namsen in Norway. We studied genetic divergence between these two life history types by using electrophoretic data from 38 protein loci. Although allele frequencies differed significantly between the types at four loci no fixed allele frequency difference was observed. Nei's genetic distance between the life history types, however, was 0.01 a relatively high value for Atlantic salmon populations. The genetic differences observed between anadromous and nonanadromous salmon accounted for 18.3% of the total gene diversity. The five nonanadromous salmon samples formed two genetically different groups corresponding to the upper and lower ranges of the nonanadromous salmon in the river. The average observed heterozygosity was 3.1% for anadromous and 1.0% for nonanadromous salmon. The low heterozygosity in nonanadromous salmon probably reflects a strong founder effect. Stockings of large numbers of anadromous salmon fry to the upper reaches of the River Namsen have caused no detectable hybridization with nonanadromous salmon. Also downstream migrating nonanadromous salmon have apparently not made any important genetic contribution to the anadromous fish.

Des saumons atlantiques, *Salmo salar*, anadromes et non anadromes coexistent dans la rivière Namsen, Norvège. Nous avons étudié les divergences génétiques chez ces deux types au moyen de l'électrophorèse de 38 loci sur des protéines. Même si les fréquences d'apparition des allèles différaient dans un rapport significatif entre les types pour quatre loci, aucune différence constante dans la fréquence d'apparition des allèles n'a été observée. La distance génétique de Nei entre les deux types était cependant de 0,01, soit une valeur relativement forte pour les populations de saumons atlantiques. Les différences génétiques entre les saumons anadromes et les saumons non anadromes expliquent 18,3 % de la diversité génétique totale. Les cinq échantillons de saumons non anadromes formaient deux groupes génétiquement différents qui correspondaient à la partie inférieure et à la partie supérieure de la plage de variations observées chez les saumons non anadromes de la rivière. L'hétérozygotie moyenne était de 3,1 % dans la sous-population anadrome et de 1,0 % dans la population non anadrome. La faible hétérozygotie chez cette dernière sous-population traduit probablement l'influence accentuée de la souche originale. L'ensemencement du cours supérieur de la rivière Namsen avec un grand nombre d'alevins de type anadrome n'a provoqué aucune hybridisation détectable avec le saumon non anadrome. En outre, les saumons non anadromes qui migraient vers le cours inférieur de la rivière n'ont apparemment pas apporté de contribution génétique importante à la population de saumons anadromes.

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The Atlantic salmon *Salmo salar* is usually anadromous but some nonanadromous populations in Europe and North America (MacCrimmon and Gots 1979) complete their whole life cycle in freshwater. The River Namsen (Fig. 1) is one of the few known cases where both anadromous and nonanadromous Atlantic salmon co-occur in the same watershed (Payne 1974; Berg 1985; Courturier et al. 1986; Hutchings 1986). Anadromous salmon inhabit the lower course of the river up to the Aunfoss waterfall which prevents upstream migration. The upper reaches of the river are inhabited by nonanadromous salmon, which area is divided by impassable Trongfoss and Sagfossen falls. The former is in the main river and the latter at the mouth of the River Mellingselva.

The nonanadromous salmon is locally known as "småblank." This Norwegian name pertains to a dwarf salmon (usually below 20 cm, maximum length 35 cm) that instead of smolting and migrating into a connected lake spend their whole life in a river (Berg 1984a, 1985). However, some of the River Namsen fish do smolt, and migrate downstream to the area inhabited by anadromous salmon (Berg 1984b). The isolation of "småblank" from anadromous salmon probably occurred about 9500 yr ago, when the Trongfoss waterfall was formed by an isostatic land uplift (Berg 1984a).

From 1950 through 1976 20 000 – 100 000 Namsen anadromous salmon fry were stocked annually between Aunfoss and Sagfossen falls. These stocked fish could have contributed genes to the nonanadromous salmon population, since male Atlantic salmon commonly mature as parr during their fresh-

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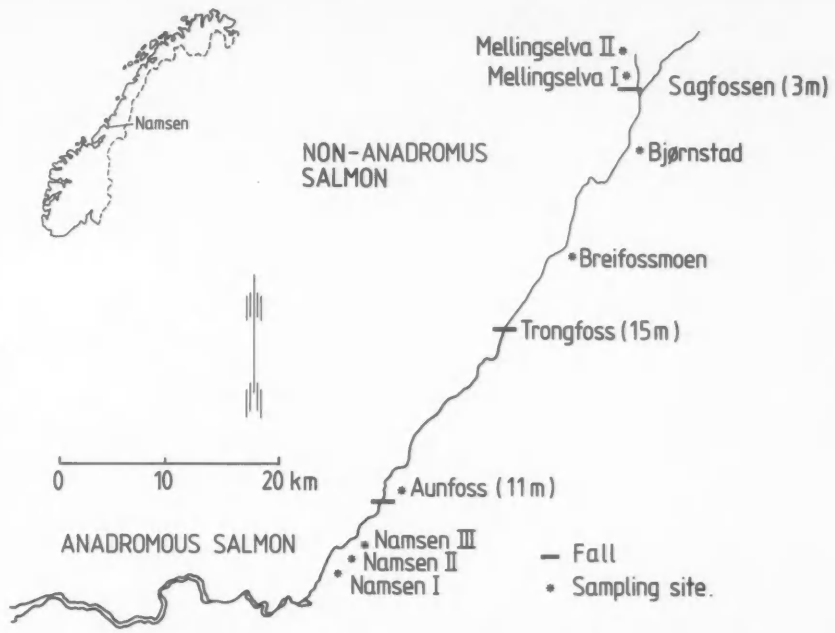

FIG. 1. Locations of the sampling sites in the River Namsen, Norway. Horizontal lines indicate impassable waterfalls and the numbers in brackets their direct vertical fall.

water residency (Jones 1959). Only a population in a tributary, the River Mellingselva, has for certain remained unaffected by these introductions because of a 3-m, impassable vertical falls.

Our objectives were to measure genetic divergence between anadromous and nonanadromous salmon in River Namsen and among nonanadromous fish isolated by impassable falls. Potential hybridization caused by stockings was examined by comparing gene frequencies in samples from the isolated Mellingselva and the main river populations. In addition, we examined possible genetic impacts of downstream straying of freshwater salmon on the anadromous salmon.

Materials and Methods

During August 1986, 71 anadromous salmon were collected from three localities by electrofishing and 246 nonanadromous salmon from five localities using gillnets (Fig. 1). We attempted to obtain samples from as large an area and as many year–classes as possible. Anadromous salmon were zero to three winters old presmolts and nonanadromous salmon varied between two and eight winters old. Fish were frozen and stored at −40°C for 7 mo before electrophoretic analysis.

Tissue samples of eye, liver, and muscle were analyzed by horizontal starch gel electrophoresis. Two buffer systems were applied, a discontinuous lithium hydroxide-boric acid buffer of pH 8.1 (Ridgway et al. 1970) or an amine–citrate buffer of pH 6.1 (Clayton and Tretiak 1972). The staining of the enzymes followed the recipes of Allendorf et al. (1977) with some minor modifications. *Sdh* was assayed using a stain buffer of pH 7.1 (d'Apollonia and Anderson 1980), and *Gpi* and *Pgm* were stained using NAD-dependent G-6-Pdh and thus NAD instead of NADP (Buth and Murphy 1980). Enzyme loci and alleles have been designated using the nomenclature of Allendorf and Utter (1976).

The following 15 enzymes encoded by 38 loci in parentheses were scored: aspartate aminotransferase (*Aat*-1,2,3, m*Aat*-1,2), alcohol dehydrogenase (*Adh*), alpha-glycerophosphate dehydrogenase (*Agp*-1,2,3), esterase (*Est*-1,2), glucose phosphate isomerase (*Gpi*-1,2), creatine kinase (*Ck*-1,2,3), isocitrate dehydrogenase (*Idh*-1,2,3), lactate dehydrogenase (*Ldh*-1,2,3,4,5), malate dehydrogenase (*Mdh*-1,2,3,4), malic enzyme (*Me*-1,2,3), phosphoglucomutase (*Pgm*-1,2), 6-phosphogluconate dehydrogenase (*Pgd*), sorbitol dehydrogenase (*Sdh*-1,2), superoxide dismustase (*Sod*), and xanthine dehydrogenase (*Xdh*). The allele frequency estimates are based on direct counts. The infrequent variation at duplicate *Mdh*-3,4 was arbitrarily assigned to *Mdh*-3. Fit of phenotypic proportions to Hardy–Weinberg expectations was tested by calculating exact probabilities using Vithyasai's method (1973). Heterogeneity of allele frequencies among samples was tested using contingency table Chi-square test. Overall genetic differences between samples were expressed as genetic distances (Nei 1972) and summarized in a dendrogram constructed using UPGMA cluster analysis. The total amount of genetic variation detected was partitioned into differences between the two life history types and subdivision among nonanadromous salmon using a hierarchical gene diversity analysis (Nei 1973). The programs of Swofford and Selander (1981) and Chakraborty et al. (1982) were employed for calculations.

Results

We detected variation only at *Aat*-3, *Mdh*-3, *Me*-2, and *Sdh*-1 (Table 1). The variant alleles we detected have been described previously (Cross and Ward 1980; Ståhl 1981, 1987) with the exception of *Mdh*-3(75). Only one of the 18 tests made for deviations from Hardy–Weinberg proportions indicated a significant deviation (Namsen II, *Aat*-3, $P = 0.03$). This single

TABLE 1. Allele frequencies at four variable loci and observed mean heterozygosities in Atlantic salmon from River Namsen.

Locality	No.	*Aat*-3 (100)	*Aat*-3 (50)	*Aat*-3 (25)	*Mdh*-3 (100)	*Mdh*-3 (80)	*Mdh*-3 (75)	*Me*-2 (100)	*Me*-2 (125)	*Sdh*-1 (100)	*Sdh*-1 (−50)	H% (38 loci)
Anadromous												
Namsen I	17	0.912	0.059	0.029	0.971	0.029	0	0.471	0.529	0.500	0.500	3.6
Namsen II	17	0.941	0.059	0	0.971	0	0.029	0.529	0.471	0.647	0.353	2.3
Namsen III	37	0.932	0.041	0.027	0.986	0	0.014	0.500	0.500	0.486	0.514	3.1
Nonanadromous												
Aunfoss	60	1	0	0	1	0	0	0.125	0.875	0.008	0.992	0.5
Breifossmoen	35	1	0	0	1	0	0	0.157	0.843	0	1	0.7
Bjørnstad	60	1	0	0	1	0	0	0.417	0.583	0	1	1.1
Mellingselva II	58	1	0	0	1	0	0	0.405	0.595	0	1	1.4
Mellingselva I	33	1	0	0	1	0	0	0.409	0.591	0	1	1.5

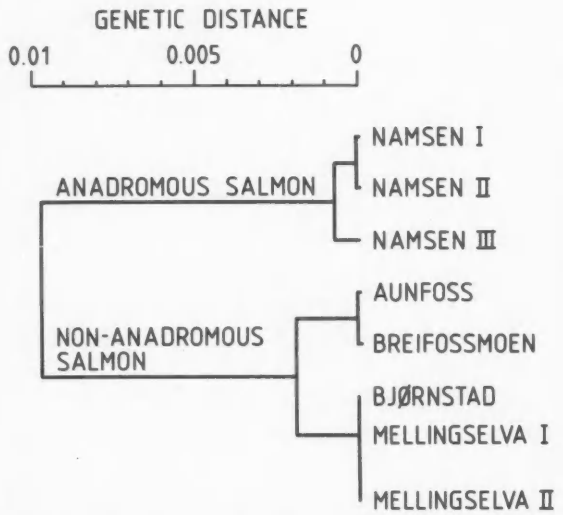

FIG. 2. Dendrogram summarizing the genetic relationships of anadromous and nonanadromous Atlantic salmon samples studied. Nei's (1972) genetic distances are based on data for 38 loci.

deviation may represent nonrandom mating or more likely a chance departure from conformity due to the number of tests performed, small sample size and the low frequency of the variant allele.

Though no diagnostic loci were found between anadromous and nonanadromous Atlantic salmon they have significantly different allele frequencies at every variable locus. The most striking difference is at *Sdh*-1. Anadromous salmon have a frequency of *Sdh*-(100) between 0.49 and 0.65 while this allele is almost absent from nonanadromous salmon (Table 1). At *Me*-2 anadromous fish have a lower frequency of allele 125 than nonanadromous salmon, and *Aat*-3 and *Mdh*-3 varied only in the anadromous salmon (Table 1). Multiple samples from anadromous salmon have statistically homogenous allele frequencies. In contrast, allele frequencies in nonanadromous salmon samples differed significantly at *Me*-2 ($\chi^2 = 41.5$; $P < 0.001$), because of the lower frequency of allele 125 in the upstream (Mellingselva and Bjørnstad) than the lower stream (Breifossmoen and Aunfoss) samples. Overall genetic differences between samples are summarized in a dendrogram (Fig. 2). Nei's (1972) genetic distance between anadromous and nonanadromous salmon is 0.01, a relatively high value for Atlantic salmon populations.

The hierarchical gene diversity analysis (Table 2) reveals the following features. Of the total relative gene diversity detected, 78.6% is attributable to within sample variation. The remaining variation is mostly due to differentiation between anadromous and nonanadromous salmon (18.3%). A smaller but still significant component (2.5%) is attributable to differences between nonanadromous salmon in the upper and lower reaches.

The 3.1% average observed heterozygosity for the anadromous salmon is comparable with that observed for 15 other wild populations from the eastern Atlantic Ocean (Ståhl 1987). The nonanadromous salmon, however, have only one third (1%) the heterozygosity of anadromous salmon.

Discussion

The question of potential hybridization between stocked anadromous and native nonanadromous fish was the primary

TABLE 2. Distribution of gene diversity at variable loci among anadromous and nonanadromous Atlantic salmon in River Namsen.

	Absolute gene diversity		Relative gene diversity (%)			
Locus	Total	Within samples	Within samples	Between morphs	Between regions within morphs	Between samples within regions
Aat-3	0.052	0.050	96.0	3.7	0	0.3
Mdh-3	0.018	0.017	98.1	1.1	0	0.8
Me-2	0.469	0.429	91.3	3.9	4.6	0.1
Sdh-1	0.326	0.184	56.4	42.3	0	1.2
Average	0.216	0.170	78.6	18.3	2.5	0.5
SE	0.109	0.093	12.6	13.8	1.7	0.4

impetus for this research. The strongest evidence against hybridization is that variant alleles at *Aat*-3, *Mdh*-3, and *Sdh*-1 in anadromous salmon are totally lacking from nonanadromous salmon populations, with the exception of a single *Sdh*-1(100) allele in the Aunfoss sample. Moreover, samples from the adjacent sites of Bjørnstad and Mellingselva, the former being stocked and the latter considered to have a natural population, have homogenous allele frequencies. This indicates that gene flow from stocked anadromous to nonanadromous salmon, if any, must have been low. Therefore, we conclude that "småblank" in the River Namsen are not hybridized with anadromous salmon.

Our results do not support the view of Hutchings and Myers (1985) who stated, based on mating experiments between dwarf nonanadromous and anadromous salmon, that interbreeding can be expected when anadromous salmon are introduced into areas formerly occupied only by nonanadromous fish. Courturier et al. (1986), however, observed reproductive segregation between co-occurring anadromous and nonanadromous Atlantic salmon and suggested that genetic differentiation existed between them. Payne (1974) and now we have reported distinct allele frequency differences between anadromous and nonanadromous salmon living in the same watershed suggesting reproductive isolation.

Unlike the anadromous samples, our nonanadromous salmon samples appear to have come from genetically distinct groups; three upstream samples (Bjørnstad and two from Mellingselva) and two lower stream samples (Breifossmoen and Aunfoss). Thus, the nonanadromous salmon are divided into at least two different demes in Namsen River. This substructuring of nonanadromous salmon exists despite the absence of a migration barrier between Bjørnstad and Breifossmoen. The subdivision, therefore, may be due to a strong selection pressure for maintenance of position in rivers above waterfalls, as outlined by Jonsson (1982) for resident brown trout *Salmo trutta*.

The low heterozygosity in the Namsen nonanadromous salmon may reflect a founder effect (i.e. small number of ancestors in a population). The Trongfoss waterfalls formed some 9500 yr ago and probably isolated anadromous salmon juveniles upstream. Most of these fish probably smolted and migrated to sea, but a few may have matured in the river. Salmonids are known to have some life history variation within populations and anadromous Atlantic salmon males frequently mature as parr (Myers 1984). Among females, however, maturation without a marine phase is rare (Bagliniere and Maisse 1985, and references therein). Therefore, we conclude that few females probably contributed to the original nonanadromous salmon population in River Namsen.

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Influence of Parr Maturity on Emigration of Smolting Atlantic Salmon (*Salmo salar*)

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Sexual maturity of parr reduces the probability of a future seaward migration. In release experiments in two separate years with Atlantic salmon in the Imsa River, Norway, immatures migrated sooner and in significantly higher proportions ($P < 0.001$) than did previously mature males. Furthermore, higher proportions of 2-yr olds than of 1-yr olds migrated, and 86–92% of the descent occurred at night. Large 2-yr olds migrated before smaller ones. Among those which did not migrate, some (3.2% of those released in 1986) were recaptured in the autumn, of which 91.9% were mature males. At Lussa, Scotland, 5.6 and 5.9% of smolting fish released in two separate years remained resident at the release site throughout the summer, and 91.8 and 93.4% of these matured in the autumn of the release year.

La maturité sexuelle des tacons réduit la probabilité d'une éventuelle migration vers la mer. Lors de remises en liberté de saumons atlantiques dans la rivière Imsa, Norvège, en deux années distinctes, les sujets immatures ont migré plus tôt et dans une proportion significativement supérieure ($P < 0{,}001$) que les mâles ayant atteint la maturité. De plus, les sujets de deux ans ont migré en proportion supérieure aux sujets de un an et 86–92 % de l'avalaison s'est produit la nuit. Les gros sujets de deux ans ont migré avant les autres. Parmi ceux qui n'ont pas migré, un certain nombre (3,2 % de ceux remis en liberté en 1986) ont été recapturés en automne; 91,9 % de ceux-là étaient des mâles à maturité. A Lussa, Écosse, 5,6 et 5,9 % des tacons en migration remis en liberté en deux années différentes ont continué à habiter au point où ils ont été remis en liberté l'été; 91,8 et 93,4 % d'entre eux avaient atteint la maturité à l'automne de leur mise en liberté.

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Typically Atlantic salmon (*Salmo salar*) emigrate from their natal river as smolting juveniles, mature after 1–4 yr in the sea at a relatively large body size, and migrate back into freshwater to spawn. After spawning, survivors emigrate to sea once again (Jones 1959). Within each population, however, a variable proportion of males matures as parr in freshwater without having been at sea (Jones 1959). This phenomenon has been interpreted as paedomorphosis (Berg 1937; Balon 1968, 1980; Thorpe 1982, 1984). The mature parr males spawn together with sea-run fish, and they are fully capable of fertilizing the eggs (Jones and King 1950, 1952; Thorpe and Morgan 1980; Hutchings and Myers 1985).

The salmon's major behavioural adaptation in freshwater is the ability to maintain station in a river and resist being swept out to sea (Arnold 1974). It acquires such abilities during its early development, and again at spawning, but to emigrate at smolting it must abandon such adaptations (Thorpe 1982, 1984, 1986), as it must also after spawning, as an emigrating kelt.

Under the accelerated development regimes of hatcheries it is common to find high proportions of males maturing as parr (Leyzerovich 1973; Kazakov 1982; Saunders et al. 1982). During the winter following this maturation these males undergo morphological changes similar to those in immature smolting fish. In spring they tend to be treated as ready for release as emigrants, or ready for transfer to seawater farms. In some cases however, mature parr remature and it is possible that they become freshwater residents, as in charr (*Salvelinus alpinus*) (Nordeng 1983) and in brown trout (*Salmo trutta*) (Jonsson 1985).

In farms mature male parr are not permitted to spawn, and in spring their testes may still be ripe. It is likely, therefore, that they would behave as mature fish, with retained freshwater adaptations, and so show a reduced tendency to migrate.

As this has undesirable implications for the efficiency of rearing practices for both intensive and extensive culture of anadromous salmonids, the behaviour of such early maturing Atlantic salmon was examined experimentally. The following null hypothesis was tested: the pattern of downstream migration was the same for all fish irrespective of age, size, sex, or state of maturity.

Materials and Methods

The hypothesis was treated at two sites, Ims (Norway) and Lussa (Scotland): (1) Ims — the Imsa River flows from Lake

TABLE 1. Ims — smolting salmon released into River Imsa and recaptured 1 km downstream during the first month after release, 1986 and 1987. Proportions of male and female immature fish were estimated from separate samples which were sexed.

	1986			1987		
	Released (11 May)	Recaptured at trap (%)	Assumed nonmigrant	Released (9 May)	Recaptured at trap (%)	Assumed nonmigrant
1-yr smolts (S1)						
Immature						
Male	189	162(85.7)	27			
Female	134	87 (64.9)	47			
Mature						
Male	260	145(55.8)	115			
Total	583	394(67.6)	189			
2-yr smolts (S2)						
Immature						
Male	80	78(97.5)	2	82	77(93.9)	5
Female	197	164(83.2)	33	310	290(93.5)	20
Mature						
Male	300	143(47.7)	157	249	97(39.0)	152
Total	577	385(66.7)	192	641	464(72.4)	177

TABLE 2. Time of migration. Numbers of fish trapped by day and night, during the first 84 (1986) and 105 h (1987) after release.

	1986			1987		
Smolt type	Night 2130–0630 h (%)	Day 0630–2130 h	Total	Night 2130–0630 h (%)	Day 0630–2130 h	Total
S1 Immature						
Males	111 (76.0)	35	146			
Females	66 (94.3)	4	70			
S1 Mature						
Males	93 (94.9)	5	98			
Totals	270 (86.0)	44	314			
S2 Immature						
Males	69 (88.5)	9	78	73 (94.8)	4	77
Females	145 (91.2)	14	159	268 (92.4)	22	290
S2 Mature						
Males	124 (96.1)	5	129	87 (89.7)	10	97
Totals	338 (92.3)	28	366	428 (92.2)	36	464

Liavatn over a distance of approximately 1 km into the sea at Ims, 58°50′N and 6°E in Southwest Norway (Hansen et al. 1984). The fish were released at the outflow of the lake, and the migrants were recovered at a modified Wolf trap, catching all descending smolts, ca 100 m from the river mouth. (2) Lussa — the Lussa River flows from Lussa Loch over a distance of approximately 6 km into the sea at Peninver, 55°30′N and 5°30′W in Southwest Scotland. Fish were released into the top pool of a 10-pool ladder carrying the effluent from the experimental ocean ranching unit at the mouth of the river. The characteristics of the nonemigrant fraction of the population were estimated by emptying the ladder in late summer.

Salmon of known parentage from the two local rivers were reared at the experimental stations at Ims and Lussa. In 1986 at Ims, 1457 fish (734 1-yr old sibling (S1) and 723 2-yr old sibling (S2) smolts (Table 1)), tagged externally with individually numbered Carlin tags, were transferred from their rearing tanks on 10 May to a net pen moored in the outflow of Lake Liavatn. Two hundred and ninety seven of the immature fish (151 S1's and 146 S2's) were sampled to estimate the sex ratio among the immature fractions, and the remaining 1160 were released from the pen just downstream of the lake outflow at 2130 h on 11 May. Arrival of the emigrants at the Imsa trap was recorded at 30-min intervals during the night until 0630 h, and at 3-h intervals during the day until 2130 h, over the 84-h period following release, and then the trap was monitored at 2-d intervals.

In 1987, 641 Carlin-tagged 2-yr old sibling fish were released directly into the river just downstream of the lake outflow at 1930 h on 9 May, and the migrants were recorded at the trap as in 1986. Before release, 264 of the immature fish were sampled to estimate sex ratios.

At Lussa, 12 252 fish of several families, tagged with X-ray readable microtags (Northwest Marine Technology Inc.), were released into the ladder in June 1984, and 7718 in June 1986. In 1986 one half of the previously mature 2-yr old males had been stripped at tagging (6 mo before release), to allow comparison of the migratory behaviour of fish with full and spent

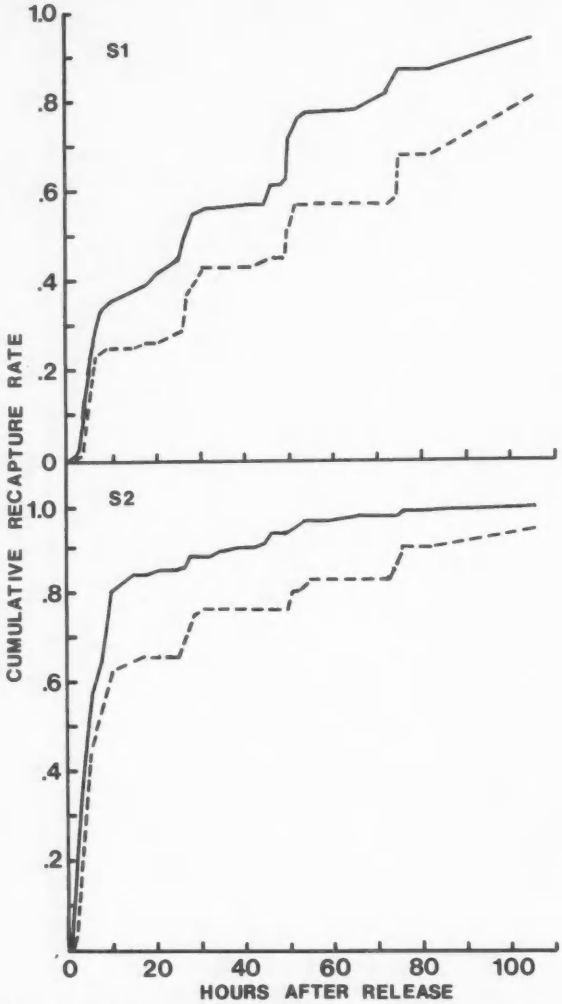

FIG. 1. Cumulative recaptures of immature (solid line), and mature male (broken line) Atlantic salmon smolts, 1 (S1) and 2 (S2) yr of age in the fish trap at the mouth of Imsa River in 1986. The smolts were released in the river 1 km above the trap.

TABLE 3. Autumn 1986; recoveries of tagged fish at Ims trap.

	S1		S2	
Smolt type	Nonmigrants in spring	Recaptured in autumn (%)	Nonmigrants in spring	Recaptured in autumn (%)
Immature				
Males	27	0	2	2 (100.0)
Females	47	1 (2.1)	33	0
Mature				
Males	115	12 (10.4)	157	22 (14.0)
Total	189	13 (6.9)	192	24 (12.5)

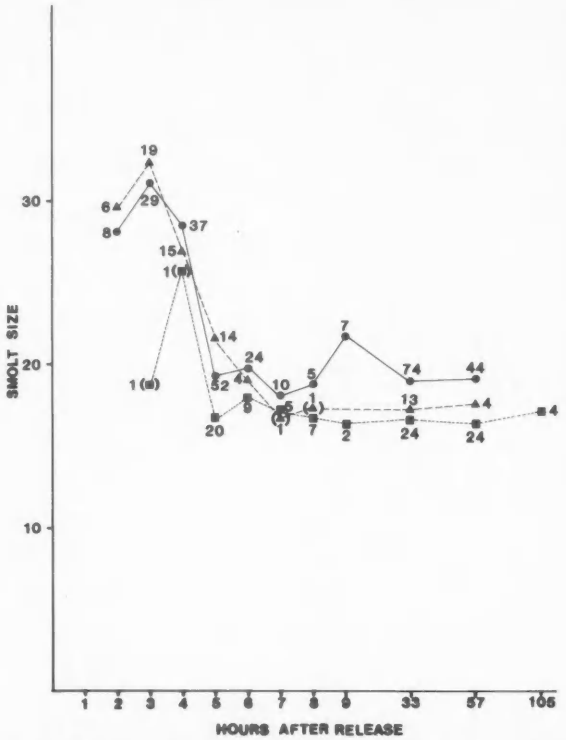

FIG. 2. Relationships between smolt size (centimetres) and time (hours) from release to recapture in the smolt trap for immature males (triangles), females (circles), and mature males (squares), released in 1987. The figures give the number of observations.

testes. The ladder was drained in October 1984, and again in October 1986, and all the fish resident there at those times were sampled to assess sex and state of maturity.

Results

Ims

In 1986, the sex ratios from the samples of immature S1's and S2's were 1.41 (58.5% males) and 0.41 (28.9% males), respectively. These figures were used to estimate the number of immature male and female smolts released (Table 1).

During the first month after release, a total of 779 migrants were taken at the Imsa trap (Table 1). Only about half of the mature males (Table 1) migrated (S1: 55.8%; S2: 47.7%), which gave significantly lower proportions of migrants than among immature males of corresponding ages (chi square tests: $P < 0.001$). The proportions of migrants among the two groups of mature males did not differ (chi square test, $P > 0.05$).

Among the immature fish, a significantly higher proportion of S2's migrated than did S1's (males: S1, 85.7%; S2, 97.5%: chi square test, $P < 0.01$; females: S1, 64.9%; S2, 83.2%: $P < 0.001$).

Over the first 84 h after release significantly more fish emigrated by night than by day (S1's and S2's pooled) (t test, $P < 0.001$) (Table 2). However, during the daylight period, there was significantly more movement among S1's (14.0%) than among S2's (7.7%).

TABLE 4. Resident salmon in the Lussa ladder in October, following their release as smolting fish in the previous June. In late autumn 1985, 50% of mature male parr were stripped of milt and tagged differentially from the unstripped mature males.

Smolt group	October 1984			October 1986			
	No.	Mature (%)	Immature	No.	Mature (%) Stripped	Mature (%) Unstripped	Immature
S1							
Males	288	282 (97.9)	6	52	—	48 (92.3)	4
Females	32	15 (46.9)	17	8	—	—	8
Total	320	297 (92.8)	23	60	—	48 (80.0)	12
S2							
Males	283	269 (95.1)	14	371	157 (42.3)	199 (53.6)	15
Females	78	59 (75.6)	19	22	—	—	22
Total	361	328 (90.9)	33	393	157 (39.9)	199 (50.6)	37
Total							
Males	571	551 (96.5)	20	423	157 (37.1)	247 (58.4)	19
Females	110	74 (67.3)	36	30	—	—	30
Total	681	625 (91.8)	56	453	157 (34.7)	247 (54.5)	49

Mature males descended more slowly than immature fish within each age–group (Fig. 1; Kolmogorov–Smirnov test: S1, $P < 0.01$; S2, $P < 0.01$). Each category of S2's descended faster than did S1's (Kolmogorov–Smirnov test: immatures, $P < 0.001$; mature males, $P < 0.01$).

Of the fish unaccounted for in spring, and assumed to be nonmigrant, 6.9% of the S1's and 12.5% of the S2's were recovered in the trap in autumn 1986 (Table 3). Of these, 92.3 and 91.7% were rematuring male parr of ages 1+ and 2+, respectively, suggesting nonmigration was usually associated with rematuration.

In 1987, the sex ratio (M/F) of immature S2's released was 0.26 (20.9% males). Seventy-seven (93.9%) immature males descended to the trap, but only 97 (39.0%) of the mature males (Table 1). As in 1986, significantly more fish (92.2%) migrated by night than by day (Table 2). Larger fish migrated sooner than smaller ones (Fig. 2).

We conclude that the degree and rate of downstream migration of mature male fish was clearly less than that of immature fish, and that greater proportions of all categories of S2's migrated than did S1's.

Lussa

The data at Lussa were examined to determine the degree of downstream migration of fish released there, and the relative rate of emigration of mature males with full or spent testes. In October 1984, of the 681 fish (5.6% of the original release) that were found still in the ladder carrying the effluent from the rearing site, 91.8% were maturing. Five hundred and seventy one of these (83.8%) were males (Table 4), of which 551 (96.5%) were maturing (97.9 and 95.1% of the S1 and S2 males, respectively). Of the 110 females, 46.9 and 75.6% of the S1's and S2's, respectively were maturing.

In October 1986, of the 453 fish (5.9% of the original release) that remained in the ladder, 423 (93.4%) were males (Table 4), of which 404 (95.5%) were maturing. Of these, 157 previously mature males had been stripped before release as compared with 199 which had not (binomial test $Z_c = 4.40$, $P < 0.001$). None of the 30 females were mature.

We conclude that small proportions of the populations became resident in freshwater each year, similar in magnitude to those at Ims. These were predominantly males which had matured as parr. Further, stripping the fish when ripe in autumn reduced their residency by about 20%.

The occurrence of maturing females in 1984 was surprising. Normally there may be interruptions to the course of such rapid maturation of females on entry to the sea.

Discussion

The degree and rate of downstream migration of mature fish released at Ims was clearly less than that of the immature fishes. Maturity appeared to inhibit movement, although it did not prevent it completely. Pentelow et al. (1933) and Österdahl (1969) both recorded mature males among runs of smolt migrants, but we know of no data comparing degree and rate of migration between maturity categories in smolting Atlantic salmon. In other anadromous salmonids, the proportion of mature parr which migrate may be smaller than that observed in Atlantic salmon. In Arctic charr, *Salvelinus alpinus*, Nordeng (1983) observed a reduced smolting rate among mature parr. Also in brown trout, *Salmo trutta*, most mature parr seem to remature and become freshwater residents rather than smolting and migrating to sea (Jonsson 1985; T. Bohlin, pers. comm.).

Greater proportions of both sexes of immature S2's migrated during the initial month than did S1's, confirming Hansen and Jonsson's (1985) earlier results. It appears to be a general feature that older individuals show circannual developmental changes earlier than younger ones (Gwinner 1986). For example, in Arctic charr and brown trout, repeat migrants start their downstream migration in spring about 2 wk earlier than first time migrants (Nordeng 1977). In Atlantic salmon, old, large smolts descend earlier in the spring than younger, smaller individuals (Österdahl 1969). The S2's in the present experiment may thus have reached a more advanced stage of smolting than the S1's at the time of release.

The predominance of nocturnal rather than diurnal movement of smolts supports previous reports (Österdahl 1969; Solomon 1978; Thorpe and Morgan 1978; Tytler et al. 1978; review in Northcote 1984). Experimentally, it has been shown that this is a light regulated behaviour which probably reduces the risk of being preyed upon (Hansen and Jonsson 1985).

The present study shows that a part of the mature males smolt and emigrate, and that another part remature as residents. Improved feeding opportunities at sea, with increased body size, increased fecundity, and supposed increased reproductive success are assumed to be the ultimate advantages of salmon emigration (Northcote 1978). However, if maturity can be achieved in freshwater before the emigratory stage is reached, then the ultimate value of that emigration is reduced. In fact the reproductive success of the mature parr in nature may equal that of the migratory males (Gross 1984, 1985), although we do not have any direct evidence for this in Atlantic salmon, yet.

Smolting and sexual maturation may be physiologically contradictory. Landgrebe (1941) maintained that there is no physiological link between the two processes. However, recent experimental evidence from several salmonid species suggests that maturation and smolting may be mutually inhibitory (Thorpe 1987). Usually the fish will not mature in the year in which they smolt. Only a very few exceptions to this are known (Nordeng 1983; Elliott 1985; Jonsson 1985; Hansen et al. 1987).

As noted earlier, hatchery practice with accelerated development regimes favours the production of mature male parr (Thorpe 1986). Since such parr show a reduced tendency to migrate, then in both enhancement and sea-ranching programmes there is reason to avoid production practices which yield high proportions of these fish. High proportions of mature parr in ranched and stocked groups may reduce the expected exploitable yield (Myers 1984), as demonstrated recently in the Baltic (Lundqvist et al. 1988). Stripping such mature parr before release reduced residency by about 20%.

Although the numbers of female residents were small in both years, 67% of these maturing at Lussa in 1984 was unexpected. Mature female parr have been reported from open river systems in France (Prouzet 1981; Bagliniere and Maisse 1985) and early maturity at small body size is characteristic of many landlocked populations (Sutterlin and MacLean 1984). Bagliniere and Maisse (1985) noted that the proportion of maturing female parr in the River Scorff was variable from year to year, and related to temperature conditions. Particularly favourable developmental conditions during winter 1983–84 may have initiated maturation in an inherently fast developing fraction of this Lussa population.

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Relationship between Anaerobic Sediment Phosphorus Release Rates and Sedimentary Phosphorus Species

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Ostrofsky, M. L., D. A. Osborne, and T. J. Zebulske. 1989. Relationship between anaerobic sediment phosphorus release rates and sedimentary phosphorus species. Can. J. Fish. Aquat. Sci. 46: 416–419.

Rates of anaerobic phosphorus release from the sediments of 57 lakes of varying trophy and sediment characteristics were measured under controlled laboratory conditions to test the hypothesis that internal phosphorus loading was a function of sediment phosphorus species. Simple regression analyses indicated that no single species can predict more than 18% of the variability in release rates. Multiple regression analysis using both water and sediment characteristics were able to explain 51% of the variation. A model based on stepwise multiple regression analysis suggests that lakes most prone to high rates of internal phosphorus loading will be alkaline with high concentrations of spring overturn total phosphorus, and with sediments characterized by high concentrations of iron-bound phosphorus, and low concentrations of organic matter.

Les taux de dégagement anaérobie de phosphore contenu dans les sédiments de 57 lacs à différents niveaux trophiques et ayant différentes caractæristiques sédimentaires, ont été mesurés en laboratoire pour tester l'hypothèse selon laquelle la charge interne de phosphore est fonction de l'espèce sous laquelle est trouvé le phosphore dans les sédiments. Les analyses de régression simple ont indiqué qu'aucune espèce unique ne peut expliquer plus de 18% de la variabilité observée dans les taux de dégagement. L'analyse de régression multiple qui tient compte des caractéristiques de l'eau et de celle des sédiments a permis d'expliquer 51% des variations. Un modèle fondé sur une analyse de régression multiple par étape donne à penser que les lacs les plus sujets à avoir une charge interne élevée en phosphore sont alcalins et contiennent du phosphore total en concentration élevée lors du turnover printanier; leurs sédiments sont également caractérisés par de fortes concentrations de phosphore lié au fer et des faibles concentrations de matières organiques.

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There have been numerous instances of successful lake restorations resulting from lake management practices that emphasize the reduction of phosphorus sources to the system (e.g. Edmondson 1970). Often, however, lakes are slow to respond and phosphorus reduction strategies have not yielded the anticipated results (e.g. Welch 1977; Larsen et al. 1979; Ryding 1981). In many cases, these are lakes that receive only a small fraction of their total phosphorus load from manageable external sources. Their major source of phosphorus is recycled from within the lake in a process that has come to be known as "internal loading."

The majority of internal phosphorus load is presumed to originate from the sediments. The prevailing mechanistic paradigm is release of iron-bound phosphorus following iron reduction although other sedimentary processes may contribute dissolved phosphorus (e.g. remineralization, pH facilitated desorption, apatite dissolution, etc.). If the hypolimnion is anaerobic, dissolved phosphorus in the pore water migrates by simple diffusion into the overlying water.

Nürnberg et al. (1986) have shown significant correlations between spring total phosphorus concentration, [TP], and the rate of phosphorus release from the sediments of 56 lakes compiled from the literature. However, these relationships do not explain much of the variation in release rates among the lakes. We wish to address two of a variety of reasons for this lack of predictive power. The first is that phosphorus release rates may be modified by such lake-specific conditions as sediment temperature, benthic activity, turbulence, etc. The second is that rates may be a function of characteristics other than, or in addition to, [TP]. In this paper we test the hypothesis that increases in explained variance in rates of phosphorus release rates will result from standardizing measurement conditions and by using sediment phosphorus species as independent variables.

Methods

We measured phosphorus release from the sediments of 57 lakes representing a variety of sediment types and trophic conditions. Lake sediments were collected from a single location at the deepest point in each lake using a Wildco K.B. gravity corer. Sediment cores were extruded in the field, and the top 2.5 cm were collected in plastic bags and held at 4°C until analysis could be started (always within 1 wk). The determination of phosphorus species, iron, and organic content of the sediments followed methods outlined in Ostrofsky (1987). [TP] and alkalinity were measured in the field or taken from published sources (see Ostrofsky (1987) for references).

To measure potential rates of phosphorus release from lake sediments, 25 cm^3 of sediment were placed in dialysis tubing (25 000 MWCO, 34 mm flat width). The contained sediment was then placed in a 300-mL erlenmeyer flask with 200 mL of distilled water. The flask was sealed with rubber cement and a

TABLE 1. Summary statistics for lake and sediment characteristics of the 57 lakes examined. [TP] in $\mu g \cdot L^{-1}$, alkalinity in $meq \cdot L^{-1}$, all sedimentary phosphorus species in $\mu gP \cdot g^{-1}$ sediment dry weight, iron in $\mu g\ Fe \cdot g^{-1}$ sediment dry weight and loss on ignition (LOI) in percent of dry weight. Notation for inorganic phosphorus species follows Ostrofsky (1987): NH_4Cl-P is loosely sorbed + interstitial P; NaOH-P is iron (and aluminum) bound P; and HCl-P is calcium-bound P.

Parameter	Mean	Range	SD
TP	22	5–111	18
Alkalinity	1.07	0.11–2.87	0.73
Total sediment phosphorus	2636	1342–9212	1193
Organic phosphorus	1477	677–2520	376
Inorganic phosphorus	1158	308–7325	1097
NH_4Cl-P	69	0–872	131
NaOH-P	832	188–5879	896
HCl-P	258	75–709	144
Sediment iron	46 547	22 000–108 000	17 269
LOI	27	10–54	10

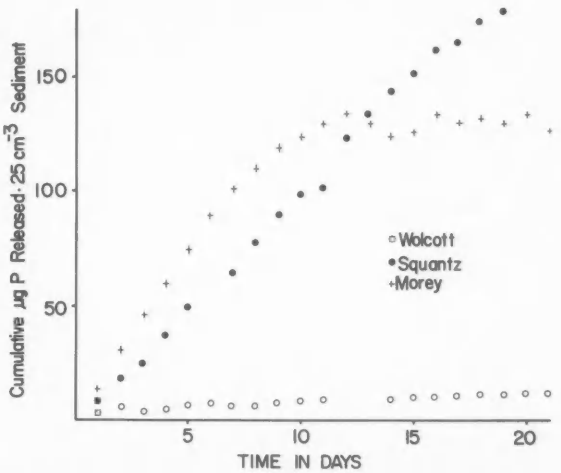

FIG. 1. Representative patterns of phosphorus release from sediments contained in dialysis membranes. Lake Morey, Vermont demonstrates the typical hyperbolic pattern of release. Squantz Pond, Connecticut demonstrates release which does not approach a horizontal asymptote in the 21-d incubation period, and Wolcott Pond, Vermont demonstrates the "all asymptote" pattern.

stopper which allowed both periodic purging with nitrogen gas and regular sampling. All samples were incubated at 10°C for 21 d. We believe that experimental design allowed us to standardize the rates of phosphorus release from a variety of environments so that differences in release may be solely attributed to differences in the sediment type. Preliminary experiments with standard phosphorus solutions indicated that phosphorus readily diffused out of dialysis bags and rapidly (~50% $\Delta P \cdot d^{-1}$) established equilibrium with the distilled incubation water.

Water samples were withdrawn by pipet from the sampling port daily, and flasks were purged with nitrogen gas during and after each sampling to maintain anoxic conditions. Total phosphorus was determined using the heteropoly blue technique (Strickland and Parsons 1968) following persulfate oxidation (Menzel and Corwin 1965). The mass of phosphorus released into the water (corrected for volume changes due to sample withdrawal) was graphed as a function of incubation time. The slope of the resultant line was taken to be the release rate. Since release rates decreased with time, or with increasing phosphorus concentration in the water, regressions were run only on the initial linear portion of the curve. Release rates were based on 7–21 observations for each lake's sediment. Simple and multiple regression analyses were done using all phosphorus species and combinations of species as independent variables.

Results and Discussion

Lake and Sediment Characteristics

Lake and sediment characteristics are given in Ostrofsky (1987). Summary statistics are given in Table 1.

Patterns of Release

When the mass of phosphorus released was graphed as a function of time, almost all lakes displayed the same pattern. Phosphorus was released at an initial constant rate (linear slope) followed by a period where the release rate decreased abruptly and approached a zero-slope asymptote. Variations from this pattern included four lakes where no asymptote was approached, that is, release rate was constant over the entire incubation period. The lakes would presumably have reached a zero-slope asymptote had the incubation time been longer. Sixteen lakes showed a pattern which was "all asymptote," that is, after some small mass of phosphorus was released in the first day or two, the subsequent release rates were not significantly different from zero. We suspect that these lakes had little releasable phosphorus. The remainder of the lakes all displayed a rectangular hyperbolic pattern of release with consistent release rates lasting for about 15 d. Representative examples of the release patterns are shown in Fig. 1

We took the slope of the initial release period to be the potential release rate of the sediment. These rates are presented in Table 2. Release rates ranged from 0.06 to 27.12 $\mu gP \cdot d^{-1}$. If we assume a dialysis bag surface of 53.56 cm^2 we can convert these rates to 0.011 – 5.064 $mgP \cdot m^{-2} \cdot d^{-1}$, values lower than, but within the range of rates reported in other studies (Nürnberg et al. 1986). However, we do not recommend this conversion because of the unknown geometric effects of containing sediments in dialysis bags and stress that the release rates measured by this technique are conservative. In-lake release rates are probably a great deal higher due to maintenance of higher sediment/water diffusion gradients by turbulence. We use the slope (SLOPE = μgP released·25 cm^{-3} contained sediment·d^{-1}) as an analog measure of phosphorus release rates in the following discussion.

Testing the Nürnberg et al. Hypothesis

Nürnberg et al. (1986) have suggested that eutrophic lakes have higher phosphorus release rates than do oligotrophic lakes. They presented two models based on literature data showing a highly significant relationship between lake [TP] and sediment release rates (RR) measured in laboratory core incubations

$$(1)\quad RR = 12.116 \log_{10}TP - 9.708 \quad n = 33,\ r = 0.504,$$

and sediment release rates measured by in situ changes in hypolimnetic phosphorus mass

$$(2)\quad RR = 13.066 \log_{10}TP - 8.961 \quad n = 43,\ r = 0.368.$$

TABLE 2. Rates of phosphorus release measured using sediments of 57 lakes from eastern North America. Units in $\mu gP \cdot 25\ cm^{-3}$ contained sediments$\cdot d^{-1}$.

Region and Lake	SLOPE
Ontario	
Brewer	0.60
Little McCauley	0.69
Found	0.06
Green	5.95
Pine	5.91
Connecticut	
Linsley	19.54
Quonnipaug	11.19
Black	1.05
Quassapaug	9.78
Taunton	9.45
Kenosia	11.88
Ball	18.70
Squantz	10.37
Bantam	8.10
Mt. Tom	17.71
Waramaug	14.57
West Side	3.90
Tyler	0.89
West Hill	3.44
Highland	3.24
East Twin	3.19
Wononscopomuc	3.92
Mudge	5.98
Wononpakook	3.92
Cream Hill	3.13
Vermont	
Dunmore	1.58
Shelburne	0.69
Iroquois	11.11
Fairfield	9.11
Carmi	11.59
Eden	0.33
Wolcott	0.34
Caspian	14.39
Shadow	14.79
Crystal	0.32
May	0.98
Echo	17.90
Island	0.49
Peacham	0.56
Ewell	6.78
Harvey's	18.14
Morey	27.12
Fairlee	5.88
Pennsylvania	
Conneaut	19.05
Crystal	13.64
Sandy	2.04
Sugar	14.96
Canadohta	15.98
Edinboro	13.47
LeBoeuf	20.89
Pleasant	23.05
New York	
Findley	14.76
Bear	18.78
Lower Cassadaga	7.73
Silver	19.61
Java	20.45
Lime	7.48

TABLE 3. Predictive relationships between lake sediment characteristics and sediment release rates (SLOPE) for 57 lakes. $*p < 0.05$, $**p < 0.01$.

Independent variable	r	SLOPE	Intercept
ln (Total sediment P)	0.311*	6.759	−43.483
ln (Organic P)	−0.021	0.384	6.527
ln (Inorganic P)	0.419**	5.147	−25.848
ln (NH_4Cl-P)	0.424**	2.528	0.469
ln(NaOH-P)	0.373**	3.851	−15.418
ln(HCl-P)	0.352**	4.949	−17.483
LOI	−0.396**	−0.280	16.997
ln(Fe)	0.370**	7.714	−73.111

In support of these models, we found a highly significant correlation ($p < 0.01$) between lake [TP] and our sediment release rates

$$(3)\quad \text{SLOPE} = 3.86 \ln \text{TP} - 1.70 \qquad n = 57, r = 0.359.$$

We also found a highly significant correlation between lake alkalinity (ALK) and sediment release rate

$$(4)\quad \text{SLOPE} = 3.75 \ln \text{ALK} + 10.11 \qquad n = 57, r = 0.424.$$

However, neither of these models explains more than 18% of the variation in release rates. A multiple regression analysis with both [TP] and ALK explained only 20%

$$(5)\quad \text{SLOPE} = 1.76 \ln \text{TP} + 2.88 \ln \text{ALK} + 4.90 \quad n = 57, r = 0.444.$$

Correlations with Sediment Characteristics

A list of sediment characteristics measured and their correlation to SLOPE is given in Table 3. Due to the log normal distribution of many of these parameters, correlation and regression analyses were done using natural log transformed data.

Many of the sediment characteristics are strongly correlated with release rates. However, none of the models presented in Table 3 yield better predictions than models based on [TP] and ALK. Most of the characteristics are strongly intercorrelated. Ostrofsky (1987) has shown that the distribution of phosphorus species in lake sediments is largely a function of iron and % LOI (loss of ignition). Incorporation of these variables into multiple regression,

$$(6)\text{SLOPE} = 5.90 \ln \text{Fe} - 0.23 \text{ LOI} - 47.51 \qquad n = 57, r = 0.480,$$

does not yield a significant improvement in predictive ability over previous equations. Incorporating all independent variables in Table 3 in a stepwise multiple regression analysis (Zar 1974), the "best" model to predict SLOPE was

$$(7)\quad \text{SLOPE} = 2.65 \ln(NH_4Cl\text{-P}) - 0.30 \text{ LOI} + 8.14 \quad n = 57, r = 0.594.$$

Nevertheless, inclusion of all variables explained only an additional 2% of the variation in SLOPE. Finally, a stepwise multiple regression with both in-lake and sediment characteristics resulted in a model which explained the most variation in release rate

$$(8)\quad \text{SLOPE} = 3.380 \ln \text{TP} + 2.274 \ln \text{ALK} + 4.270 \ln \text{NaOH-P} - 0.243 \text{ LOI} - 20.642 \qquad n = 57, r = 0.715 \quad \text{SE} = 5.342.$$

We do not present this model as a means of predicting in situ rates of phosphorus release from lake sediments. Sediment contained in dialysis tubes has a much greater surface to volume ratio than lake sediments, and the presence of the dialysis membrane has unknown effects on diffusion rates. Nevertheless, we feel that this approach has advantages. The first is very practical. Containing sediments in dialysis membranes eliminates the inevitable resuspension of fine particulates which accompanies nitrogen gas purging or stirring of waters overlying intact sediment cores (e.g. Holdren and Armstrong 1980). Our own data (unpubl.) suggest that this resuspension is a highly variable component of resultant phosphorus concentrations in water overlying cores, and can lead to serious overestimates of release rates. Dialysis membrane also allows for a more uniform treatment of different sediments.

While model (8) can not be used to estimate in situ rates of phosphorus release without field testing, the model does suggest that lakes most likely to have high rates of internal phosphorus loading will be those lakes which are eutrophic (high spring [TP]), and alkaline, with high concentrations of iron-bound phosphorus and low organic content in their sediments. Although over half of the phosphorus in lake sediments is organic-bound (Table 1), the low correlation between organic-bound phosphorus and release rates suggests that this fraction is not significantly involved in the phosphorus release measured here. Similarly, apatite phosphorus (HCl-P) does not seem to be important — its high correlation with SLOPE in Table 3 may be an artifact of its correlation with other phosphorus species in the sediments (Ostrofsky 1987). That the in-lake characteristics, [TP] and ALK, contribute to the prediction of SLOPE is somewhat of an enigma. All release experiments were done using distilled water; sediments were not in contact with lake water at all. One possible explanation is that these variables may be strongly correlated with some yet unmeasured sediment characteristics which do play an important role in phosphorus release. For example, lake alkalinity may be strongly correlated with calcium concentration in the sediments, and calcareous sediments are known to behave differently than noncalcareous sediments with respect to phosphorus release (Holdren and Armstrong 1980).

Comparisons with other Studies

Most previous work concerned with sedimentary phosphorus release rates have examined the effects of redox, mixing, microorganisms, temperature, etc. Only a few studies have attempted to identify the role of various sedimentary phosphorus species in internal phosphorus loading. Kamp-Nielsen (1974); Hosomi et al. (1981); Furumai and Ohgaki (1982); and Boström (1984) have all suggested that the concentration of iron-bound phosphorus is a most important factor in determining release rates. This observation is supported by the data described above. The role of organic phosphorus is not clear. Kamp-Nielsen (1974) and our results suggest that organic phosphorus is not a significant factor whereas Bates and Neafus (1980) and Boström (1984) suggest that it can be important. Similar conflicting observations have been made concerning calcium-bound phosphorus (Kamp-Nielsen 1974; Furumai and Ohgaki 1982). Boström's (1984) point that a simple relationship between sediment composition and phosphorus mobility probably does not exist is well taken in light of both the above anomalous conclusions in the literature, and our inability to explain more than 50% of the variation in release rates given both sediment and in-lake characteristics. It is clear, however, that oligotrophic lakes (e.g. Caspian and Shadow lakes, Vermont) can have high rates of phosphorus release from anaerobic sediments, and rapid eutrophication may result if these lakes were to develop anoxic hypolimnia.

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Extinction of a Population of Anadromous Parasitic Lamprey, *Lampetra tridentata*, Upstream of an Impassable Dam

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Beamish, R. J., and T. G. Northcote. 1989. Extinction of a population of anadromous parasitic lamprey, *Lampetra tridentata*, upstream of an impassable dam. Can. J. Fish. Aquat. Sci. 46: 420–425.

The construction of dams on the outlet of Elsie Lake, in British Columbia, prevented young adult Pacific lamprey (*Lampetra tridentata*) from going to sea and adult lamprey from spawning above the dams. This resulted in a high incidence of lamprey attacks on resident trout that was initially interpreted as indicating that the lamprey population was landlocked. This study showed that a landlocked population of lamprey was not established. The attacks on fish occurred for only a brief period each year. Attacks on trout stopped approximately 7 yr after dam construction. Because this was the average estimated age when ammocoetes metamorphosed and scarred fish or ammocoetes were no longer found, we concluded that metamorphosed lamprey were unable to survive to maturity. The inability to establish a landlocked population indicated that the transition from an anadromous parasitic life history type to a freshwater parasitic life history type did not occur as quickly or as easily as previously suggested. The study also provided evidence that the statolith method of age determination may provide accurate ages.

La construction de digues à l'exutoire du lac Elsie, Colombie-Britannique, a empêché de jeunes lamproies adultes du Pacifique (*Lampetra tridentata*) d'atteindre la mer et des lamproies adultes d'aller se reproduire en amont des digues. Cela s'est traduit par une fréquence élevée d'attaques de lamproies contre des truites résidentes, ce qui a été interprété initialement comme le signe que la population de l'endroit était devenue une population de lac. Cette étude montre qu'une telle population ne s'est pas établie. Les attaques sur les poissons se produisaient durant une brève période chaque année. Les attaques contre les truites ont cessé au bout de 7 an environ après la construction des digues. Puisque sept ans constituent l'âge moyen estimé de la métamorphose de ces ammocètes et que des poissons portant des cicatrices ou des ammocètes n'étaient plus trouvés passé cette période, il a été conclu que les lamproies métamorphosés n'avaient pas pu survivre jusqu'à maturité. L'inaptitude à constituer une population de lac indique que la transition d'un mode de vie anadrome parasitique à un mode de vie parasitique en eau douce ne s'est pas fait aussi rapidement ni aussi facilement qu'on aurait pu l'imaginer. L'étude indique également que la méthode de détermination de l'âge par les statolithes peut conduire à des résultats précis.

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The calamitous effect on commercial fisheries in the Great Lakes resulting from predation by the landlocked sea lamprey (*Petromyzon marinus*) is well known (Applegate 1950; Smith and Tibbles 1980). How the sea lamprey became landlocked is still not understood. One suggestion is that the anadromous form became landlocked after it gained access to the Great Lakes (Aron and Smith 1971; Smith 1972). Another theory is that a landlocked form was part of the original fish fauna in Lake Ontario and the Finger Lakes of New York State (Christie 1973; Morman et al. 1980). The question of how and when the freshwater feeding form originated was perceived to be of little importance relative to the devastating effects caused by the landlocked form in the upper Great Lakes. How the transition occurs from the anadromous life history type to the freshwater parasitic type is important, however, if this transition can occur quickly.

There are a number of reports that anadromous parasitic lamprey can become landlocked parasitic lamprey when access to the sea is prevented (Zanandrea 1961; Scott and Crossman 1973; McPhail and Lindsey 1970; Hubbs 1971; Hart 1973; Vladykov and Kott 1979a). These reports imply that for the anadromous parasitic lampreys in general and for the Pacific lamprey (*Lampetra tridentata*) in particular, the change to a freshwater parasitic mode of life occurs easily. If a transition to freshwater feeding can occur as a result of the construction of physical barriers such as dams, then the "landlocked" form could be an important new source of mortality to commercially and recreationally important fishes.

Specific references to landlocked forms of *L. tridentata* in: Elsie Lake (Pletcher 1963; Hart 1973), in the Columbia River (McPhail and Lindsey 1970), in the Fraser River at Prince George (McPhail and Lindsey 1970), at Cultus Lake (Vladykov and Kott 1979a), in the Pit River and Klamath River drainages (Hubbs 1971), and in Cowichan Lake (Hart 1973), all clearly imply that this species readily adapts to feeding in freshwater after metamorphosis. In British Columbia, *L. tridentata* is com-

monly found in coastal rivers and streams (Beamish, unpubl. data). During their spawning migration in freshwater, maturing adults travel many hundreds of kilometres into the headwaters of most major river systems (Beamish 1980, Beamish unpubl. data). This spawning behaviour and subsequent downstream movement of larvae distributes *L. tridentata* throughout the watershed.

In British Columbia, it is common to dam rivers as well as to build passages to provide new access for salmon to previously inaccessible river and lake habitat. Because *L. tridentata* is commonly found throughout most coastal watersheds in British Columbia and because previous reports clearly give the impression that the adult readily adapts to feeding in freshwater during its trophic phase, it is important to determine if past and planned alterations to rivers might produce landlocked populations of parasitic lamprey that could have serious effects on freshwater fishes in the future. In this report we reexamine the earlier report by Pletcher (1963) that a landlocked form of *L. tridentata* exists in Elsie Lake on Vancouver Island.

Materials and Methods

Historic fisheries data were obtained from the files of the British Columbia Fisheries Branch; from Pletcher (1963) and other records (Northcote unpubl. data). Samples of fish were obtained from Elsie Lake in March and July 1981 and in July 1987 using gillnets with stretched mesh sizes ranging from 40 to 113 mm. Fish were examined for scars or wounds or for any unusual scale growth pattern that might indicate previous scarring. In 1987, a sample of cutthroat trout (*Salmo clarki*) that represented the range of sizes obtained in the 1981 and 1987 samples was selected, and scales, pectoral fins, and otoliths were collected for age determination using the methods described in Chilton and Beamish (1982).

In 1986 and 1987, two inlets to Elsie Lake and the outlet were electroshocked. Fishing effort was concentrated in typical lamprey habitat. Ammocoetes collected from the outlet in 1986 were measured and fed in the laboratory according to the procedures described by Richards et al. (1982) to determine lengths at metamorphosis and the ability of metamorphosed lamprey to survive in freshwater. Statoliths from a range of sizes of a small sample of ammocoetes and from a sample of 11 metamorphosed lamprey collected in 1987 were used for age determination according to the technique described by Volk (1986) and Russell (1986). Ages were estimated to compare the duration of the scarring period with the time required for metamorphosis, and to determine if reproduction occurred in freshwater. Ages were estimated after statoliths were held in immersion oil for 2 wk. In most cases, annuli were distinguishable by simply viewing the side of the statolith with transmitted light while the statolith was in the oil. Because the lamprey were sampled in mid-September, much or most of the growth for the year probably had occurred. Ages from statolith analysis were assigned using a January 1 "birthday." If little or no growth occurred during the winter months, the mean length represented the size of the lamprey at the beginning of the growing season for each age indicated.

Results

Elsie Lake is located in the Ash River basin in central Vancouver Island, about 40 km northwest of Port Alberni (Fig. 1).

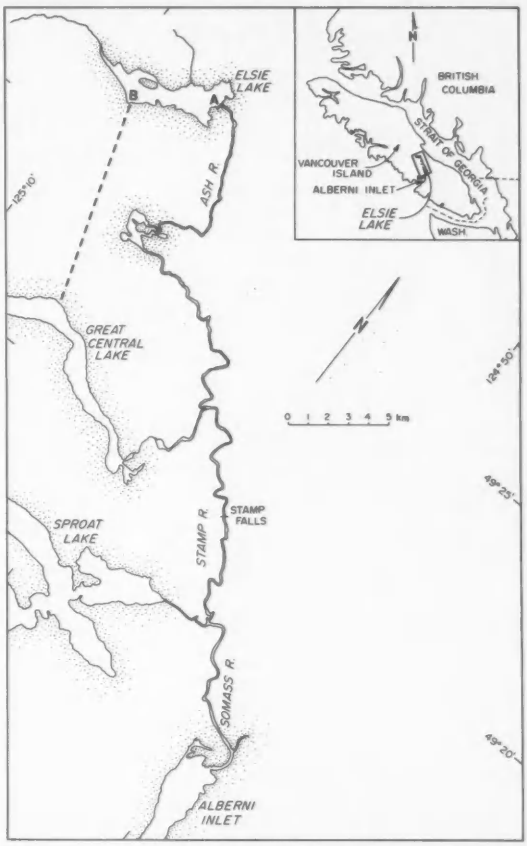

FIG. 1. Location of Elsie Lake. Dams were constructed in area of (A) outlet and (B) intake for the pipeline to Great Central Lake (broken line).

Elsie Lake is drained by the Ash, Stamp, and Somass rivers that flow into Alberni Inlet at Port Alberni.

From 1957 to 1959, five dams were constructed on the outlet and in the area immediately around the outlet. Water was diverted from the end of the lake opposite the outlet nearly 8 km to a power-generating station at Great Central Lake, 226 m below the level of Elsie Lake. The dams increased the surface area of the lake from 1.46 to 6.60 km^2. An automatic valve controlled the water flow through a 2-m diameter outlet pipe that protruded about 2 m from the "middle" of the dam, above the outlet. The dams and this new outlet completely prevented fishes from entering the lake. It was probable that this new valve controlled outlet pipe and the new intake for the generating station prevented lamprey from leaving the lake.

Trout have been collected in Elsie Lake since 1953 (Table 1). No marks were observed on any trout collected before the dams were constructed. Fish sampled in May 1959, immediately after the dams were completed, had an abundance of fresh wounds caused by lamprey. Most fish, 74.5%, had fresh wounds; only 2.1% had older wounds that were healing (Pletcher 1963). Wounds penetrated the skin but were small, averaging about 2.5 mm in diameter. They were found in almost all regions of the body and the examples shown in Pletcher (1963) indicate that there were multiple attacks. The percentage of fish with scars declined in 1960 to 59.6%, in 1962 to 40.9%, and in 1963

TABLE 1. Catches of trout in Elsie Lake from data obtained from files of the Province of British Columbia and from this study.

Date	Catch	Lamprey marks
1953 summer	7 trout (4 cutthroat 3 rainbow trout)	None
1957 September 14	66 trout (30 cutthroat 36 rainbow trout)	None
1959[a] May 20	47 trout[b]	76.6% scarred (74.5% new, 2.1% old)
1960[a] May 6, June 6	104 trout[b]	59.6% scarred (31.7% new, 27.9% old)
1962[a] July 13	110 trout[b]	40.9% scarred (27.3% new, 13.6% old)
1963[a] April 3, June 5	136 trout[b]	20.4% scarred (9.9% new, 10.5% old)
1969 August 29, October 19	174 trout (23 cutthroat, 123 rainbow trout, 18 unidentified)	None
1981 March 19, July 9	135 trout (11 cutthroat, 32 rainbow trout, 92 unidentified)	None
1987 July 8	32 trout (18 cutthroat, 14 rainbow trout)	None

[a]British Columbia fish and game data, recorded in Pletcher (1963).
[b]Species not recorded.

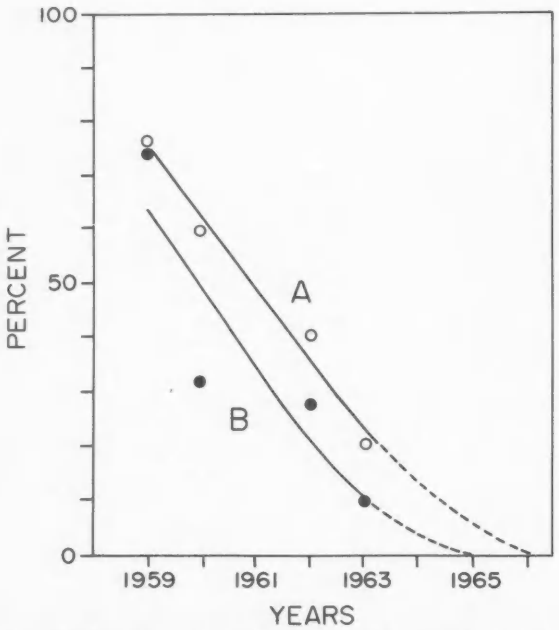

FIG. 2. Percent of trout population with scars, the regressions are linear for (arcsine) transformed proportions. (A) open circles, the percentage of trout with fresh and old scars, $y = 68.32 - 7.92x$, $n = 4$, $r = -0.99$, $F_{1,2} = 71.8$, $p = 0.01$. (B) solid circles, the percentage of trout with fresh scars, $y = 61.57 - 8.54x$, $n = 4$, $r = 0.90$, $F_{1,2} = 8.81$, $p = 0.05$.

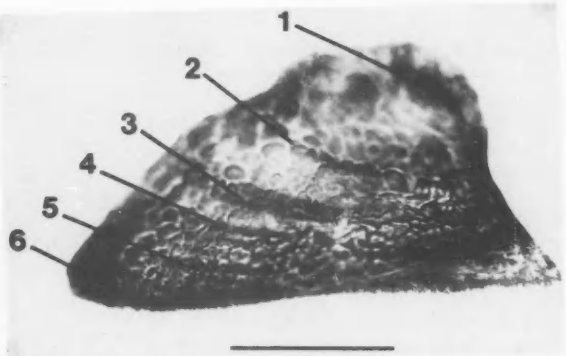

FIG. 3. Statolith from a 113-mm, metamorphosed *L. tridentata* from the Ash River, age 6+. The top of the photograph is slightly out of focus in order to focus the annuli representing the older years. Bar represents 0.1 mm.

to 20.4%. At the same time, the percentage of fish with old scars increased and ranged from about one-half of the fish in 1960 and 1963 to about one-third of the fish in 1962.

Fish were not sampled again until 1969; and no lamprey marks were found on any of the 174 trout that were collected in August, October, and November of that year. In 1981, 135 trout were collected in March and July. Again, no marks were found on any fish. The final sample of 32 trout was collected in July 1987 and again, no marks were found on any of the fish.

The relationship between the arcsine transformed yearly percent scarring incidence and time was strongly linear (Fig. 2). Using this relationship, it can be predicted that in 1964 the scarring rate would be 12.6%, 5% in 1965, and 1% in 1966. If only the fresh scarring data were used, the linear relationship indicated that the incidence of fresh scars in 1964 was 3.2%, 0.1% in 1965 and no fresh scars in 1966.

The sample of 18 cutthroat trout collected in July 1987, ranged in size from 16.2 to 47.5 cm and in age from 2+ to 7+ yr. The largest fish was 47.5 cm and age 7+ yr. Ages determined from the three structures were similar. The broken and burnt otolith method produced the most distinct annuli.

Annuli on lamprey statoliths usually were distinct for the first 3 yr (Fig. 3). Annuli were difficult to distinguish from 4 to 7 yr because they were more closely spaced and less distinct (Fig. 3). Four statoliths of the 11 recently metamorphosed *L. tridentata* collected from the Ash River on September 14, 1987 could not be aged. Ages from 7 ranged from 5+ to 7+ yr with an average age of 6+ yr. If these fish remained in freshwater

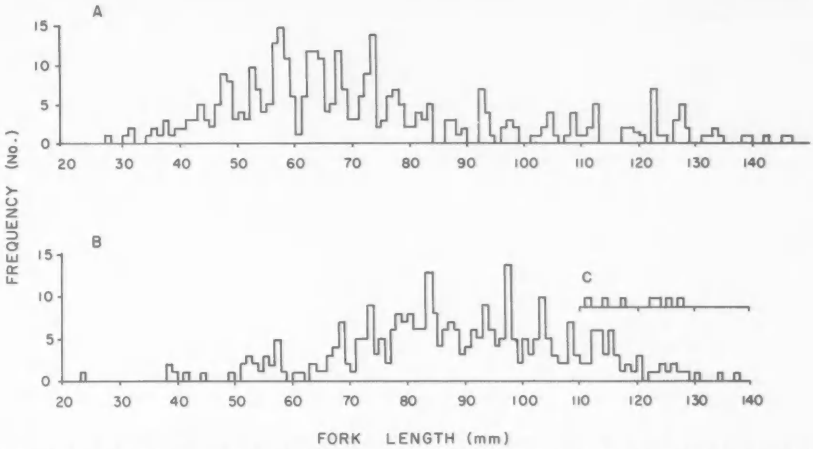

FIG. 4. Length frequency distribution of two samples of ammocoetes collected on (A) May 1, 1986; (B) September 14, 1987; and (C) the size of metamorphosed lamprey collected on September 14, 1987 from the Ash River.

TABLE 2. Year–classes contributing to the population of feeding young adult lamprey in Elsie Lake. Brackets indicate a year–class failure and the possibility of a year–class failure in 1958 is indicated by *.

	Year trout examined			
Age	1963	1964	1965	1966
8	1955	1956	1957	1958*
7	1956	1957	1958*	(1959)
6	1957	1958*	(1959)	(1960)
5	1958*	(1959)	(1960)	(1961)

until the next spring, ages would range from 6 to 8 yr and average 7 yr. These fish ranged in length from 111 mm to 127 mm (Fig. 4C) and averaged 120 mm. In 1986, 11 ammocoetes collected on May 1 and fed in the laboratory, metamorphosed at an average length of 114 mm while the remaining sample of 11 ammocoetes averaged 117 mm in total length. This remaining sample metamorphosed at an average length of 121 mm in August 1987.

The lengths of 364 ammocoetes collected on May 1, 1986 and 318 ammocoetes collected on September 14, 1987 ranged from 27 to 147 mm (Fig. 4). Ages estimated from ammocoetes using statoliths and corresponding mean lengths were as follows: 2+ yr, 62 mm; 3+ yr, 68 mm, 92 mm, 98 mm; 4+ yr, 106 mm, 92 mm, 105 mm; 5+ yr 112 mm.

Discussion

The damming of Elsie Lake isolated a portion of the population of *L. tridentata* ammocoetes that were resident in the Ash River system. Metamorphosed lamprey probably could not leave the lake resulting in confinement and freshwater feeding. The small size of the wounds indicated that the stranded young adult lamprey probably grew very little despite feeding on trout. To show that a landlocked population did not develop it was necessary to show that it is improbable that reproduction occurred. Lamprey were not found in the recent surveys, but it was not possible to survey all possible habitats. If, however, the age at which lamprey metamorphosed corresponded to the maximum predicted scarring period, then it was probable that no reproduction occurred.

Adult *L. tridentata* migrated into the Stamp River and probably into the Ash River from May to September with the largest number migrating in July (Beamish 1980, Beamish, unpubl. data). Because dam construction did not begin until the summer of 1957, the lamprey migrating upstream in the spring would have passed through Elsie Lake and spawned in 1958. The construction did block access to the lake in the late spring of 1958 and the dams were completed by July 1958. Therefore it was doubtful if any spawning occurred in 1959 because lamprey did not begin to pass through the Stamp Falls fish bypass downstream of the lake (Fig. 1) until mid-May, at the same time the outlet was being blocked. It would be impossible for upstream migrating adults to spawn above the dam in 1960. It was predicted that 3.2% of the trout would have fresh scars in 1964 but only 0.1% would have fresh scars in 1965. The predicted scarring percentage for fresh and old scars was 5.0% in 1965 and 0.7% in 1966, indicating that attacks on fish stopped in 1964 or 1965.

Rainbow trout (*Salmo gairdneri*) in Elsie Lake live up to 6 yr (Nilsson and Northcote 1981) and if the last attacks occurred in 1964 or 1965, it is unlikely that scars would be present on any of the rainbow trout sampled in 1969 which was confirmed by observation. Cutthroat trout also live up to 6 yr (Nilsson and Northcote 1981). Our age analysis of a small sample of cutthroat trout from Elsie Lake showed that most ranged from 2+ to 5+ yr with one fish 7+ yr. Again, with this age structure and with no attacks after 1964 or 1965, the sample of cutthroat trout in Elsie Lake taken in 1969 would not be expected to have lamprey scars as was observed. Thus, the last lamprey attacks probably occurred in 1964 or no later than 1965.

Lampetra tridentata begin metamorphosis in late July and migrate to sea from late fall until late spring of the next year (Beamish 1980; Richards 1980). Because the dam was completed by July 1958, lamprey metamorphosing above the dam probably would be able to leave the lake. Our estimate of age of recently metamorphosed *L. tridentata* from Ash River ranged from 5+ to 7+ yr. These lamprey would remain in the watershed above the dam until the spring when they would range

from 6 to 8 yr with most young adults age 7 yr. Russell (1986) found that the age range for metamorphosing *L. tridentata* from Babine Lake was 4+ to 7+ yr with most age 5+ yr. This corresponds to an age range in the following spring of 5 to 8 yr with the average age 6 yr. Because we aged only a small sample of young adults, we considered that the range of ages could include the age range in our study and in Russell's (1986) study. Therefore the age range of metamorphosing lamprey could be 5 – 8 yr with an average age of 6 or 7 yr.

Knowing that the last normal spawning upstream of the dam site occurred in 1957 with a possibility that 1958 spawning was affected by construction activities and that no spawning would occur in 1959, it was possible to reconstruct the year–class structure of the population of young adults that fed in the lake in 1963, 1964, 1965, and 1966 (Table 2). If most of the lamprey were age 6 and 7 yr, it was evident that recruitment of metamorphosed individuals could be reduced in 1963 as a result of a smaller 1958 year–class, further reduced in 1964 because of the 1959 spawning failure, very small in 1965 and possibly ending in 1966. The predicted decline in scarring in 1964 and 1965 and ending in 1966 is consistent with the decline in the recruitment of feeding young adults that would be expected from the estimates of age composition of metamorphosing lamprey.

We examined the possibility that a small number of young adult lamprey that fed in 1959, were able to survive and ultimately reproduce in freshwater. The possibility that confinement to freshwater might select for a few individuals that genetically were able to feed and grow to maturity in freshwater would be higher if the size of the population was large. There are no direct estimates of abundance, but the 1959 scarring rate of 76.6% indicates that lamprey could have been abundant then. Estimates of abundance of lamprey moving through an artificial fish bypass in the Stamp River (Fig. 1) ranged from 20 000 to 50 000 adult lamprey (Beamish unpubl. data). Accordingly, it appears that *L. tridentata* could have been abundant in the watershed and in the Ash River prior to 1959.

If only a small percentage of the freshwater feeding young adults survived and our age estimate at metamorphosis is correct, there would be five generations of lamprey from 1959 to 1987. Five generations that metamorphose at ages from 5 to 8 yr would produce a large number of lamprey that would easily be detected in either of the inlet streams. For example, in Babine Lake the first adult *L. tridentata* was observed in 1963 and there are presently about 7200 mature adults returning to spawn annually (Farlinger and Beamish 1984; Russell 1986). The apparent abundance of *L. tridentata* in the Ash River watershed and the inability to detect any evidence of lamprey upstream of the dam since the mid-1960s strongly suggests that not even a very small percentage of the population of young adults were adapted to feed, grow, and reproduce in freshwater.

The failure to establish a landlocked population indicates that the young adults died in freshwater. In the laboratory, it is difficult to keep metamorphosed anadromous parasitic lamprey in freshwater (Potter 1970; Potter and Beamish 1977; Beamish and Youson 1987; Clarke and Beamish 1988). Ability of *L. tridentata* to survive confinement in freshwater varies among populations, ranging from 5 to 10 mo (Clarke and Beamish 1988). A difficulty in the latter study was that most lampreys were not fed and those that were, ate very little. The amount of feeding in Elsie Lake as indicated by the scarring percentages, confirmed that even when feeding occurred, it was difficult for *L. tridentata* to survive easily in freshwater.

Because the ability to survive in freshwater varies among populations (Clarke and Beamish 1988), it is possible that *L. tridentata* from the Ash River are less able to survive than other populations. The sample of 11 *L. tridentata* that metamorphosed in the laboratory in the fall of 1986, survived until the first week of August 1987 without feeding, indicating that the Ash River population is better able to remain in freshwater than the six populations tested in the study of Clarke and Beamish (1988). We realize that we cannot use this one example to conclude that the construction of dams will not result in the establishment of a landlocked parasitic population of *L. tridentata*. However, this study, the study of Clarke and Beamish (1988) and the observation that no landlocked populations of *L. tridentata* have been found in British Columbia show that this species does not easily become landlocked. The inability of this anadromous lamprey to remain in freshwater suggests that the genetic change that needs to occur is either extremely rare or a series of changes are required that occur in sequence in response to environmental changes (Beamish 1985). Barriers or new access areas for anadromous species would not commonly result in the establishment of landlocked forms because it is unlikely that all the necessary changes would occur at one time. Nonparasitic, satellite species (Vladykov and Kott 1979b), such as *Lampetra hubbsi*, probably evolved from freshwater species and not as a consequence of barriers to migration of anadromous species (Hardisty 1986). Freshwater parasitic populations such as those described by Hubbs (1971); Bond and Kan (1973); Vladykov and Kott (1979a); and Beamish (1982) as *L. tridentata* or derivatives would develop when forms that could feed and osmoregulate in freshwater became confined to freshwater.

The duration of the period of scarring on trout also can be used to assess the accuracy of the ages determined for recently metamorphosed *L. tridentata* in Ash River. Because the estimated ages of metamorphosed lamprey were identical to the number of years that scars were predicted to occur on trout, it is probable that the ages of the postmetamorphic lamprey were a reliable estimate of the age of young adults. It is beyond the scope of this report to discuss the problems of estimating the age of lamprey. The statolith method provides a quantitative determination if it can be validated. The method has been validated in laboratory studies for younger lamprey (Medland and Beamish 1987) but it is still unknown how reliable the method is for older lamprey and for populations in the natural environment.

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Relationship of Mercury Concentrations in Lake Trout (*Salvelinus namaycush*) and Smallmouth Bass (*Micropterus dolomieui*) to the Physical and Chemical Characteristics of Ontario Lakes

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Concentrations of mercury in dorsal muscle tissue of lake trout (*Salvelinus namaycush*) from Ontario lakes were positively correlated with variables indicating lake dystrophy (dissolved organic carbon, colour, iron, transparency) and were also correlated with watershed area and lake area. Stepwise multiple regression selected dissolved organic carbon as the only variable which explained a significant amount of variation (37%) in mercury concentrations in lake trout. The relationship between dissolved organic carbon and mercury appeared to be strongest in the group of lakes with values of dissolved organic carbon less than 4.0 $mg \cdot L^{-1}$. In contrast, mercury concentrations in smallmouth bass (*Micropterus dolomieui*) were correlated with variables reflecting both water hardness (magnesium, calcium, conductivity) and acidity (pH, alkalinity). The relationship was inverse for the water hardness variables and positive for acidity. Stepwise regression identified three variables significant in explaining variation in mercury in smallmouth bass: calcium, dissolved organic carbon, and latitude. Mechanisms that may explain the effects of organic matter, water hardness, and acidity on mercury accumulation by fish are discussed.

Il existe une corrélation positive entre la concentration de mercure dans les muscles dorsaux de la truite fardée (*Salvelinus namaycush*) dans des lacs de l'Ontario et des variables indicatives de la dystrophie des lacs (teneur en carbone organique dissous, couleur, fer, limpidité); il existe également une corrélation avec la superficie du bassin hydrologique et la superficie du lac. Les régressions multiples par étapes ont montré que la teneur en carbone organique dissous est la seule variable susceptible d'expliquer une partie importante de la variation observée (37 %) dans la concentration en mercure dans les tissus de la truite fardée. Il semble que la relation entre cette variable et le mercure est la plus forte dans le cas des lacs qui ont une teneur en carbone organique dissous inférieure à 4,0 $mg \cdot L^{-1}$. Il en va autrement avec l'achigan à petite bouche (*Micropterus dolomieui*) dont la concentration en mercure dans les tissus est en corrélation avec les variables liés à la dureté de l'eau (magnésium, calcium, conductivité) et à l'acidité (pH, alcalinité). Il y a un rapport inverse entre la teneur en mercure et les variables liées à la dureté de l'eau et un rapport positif entre le mercure et l'acidité. La régression par étapes a indiqué trois variables qui agissent de façon importante sur la variation de la teneur en mercure dans l'achigan à petite bouche, soit le calcium, la teneur en carbone organique dissous et la latitude. Il est question dans l'article des mécanismes qui peuvent expliquer les effets de la matière organique, de la dureté de l'eau et de l'acidité sur l'accumulation du mercure dans les tissus des poissons.

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Large numbers of lakes in North America (Brouzes et al. 1977; Heiskary and Helwig 1986; OMOE and OMNR 1987) and Sweden (Björklund et al. 1984) distant from anthropogenic sources of contamination contain fish with mercury concentrations exceeding the 0.5 $\mu g \cdot g^{-1}$ level considered safe for human consumption (IJC 1977). The accumulation of mercury by fish in such lakes can be influenced by a variety of environmental factors, including pH (Björklund et al. 1984; Heiskary and Helwig 1986; Suns et al. 1987; Wren and MacCrimmon 1983), alkalinity (Scheider et al. 1979), hardness (Rodgers and Beamish 1983), aluminum (Heiskary and Helwig 1986), selenium (Rudd et al. 1980), water temperature (MacLeod and Pessah 1973; Reinert et al. 1974), trophic relationships present in the lake (Akielaszek and Haines 1981), watershed size relative to lake size (Suns et al. 1987), and

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atmospheric deposition (Björklund et al. 1984; Evans 1986; Johnson 1987).

Interest in the role of pH and alkalinity in mercury accumulation has increased with concern about the ecological consequences of acidic deposition. However, research in Canada has not yet determined if acidification of lakes influences mercury levels in fish of a size and species usually consumed by humans. In this study, we examine the relationship of lake acidity and other physical and chemical parameters to mercury concentrations in adult lake trout (*Salvelinus namaycush*) and smallmouth bass (*Micropterus dolomieui*) from Ontario lakes. These species were studied because of their importance to the recreational fishery in Ontario and because they have different habitat requirements.

Methods

Fish Collection and Mercury Analysis

Fish were collected during a province-wide survey of contaminants in sport fish (OMOE and OMNR 1987). In most

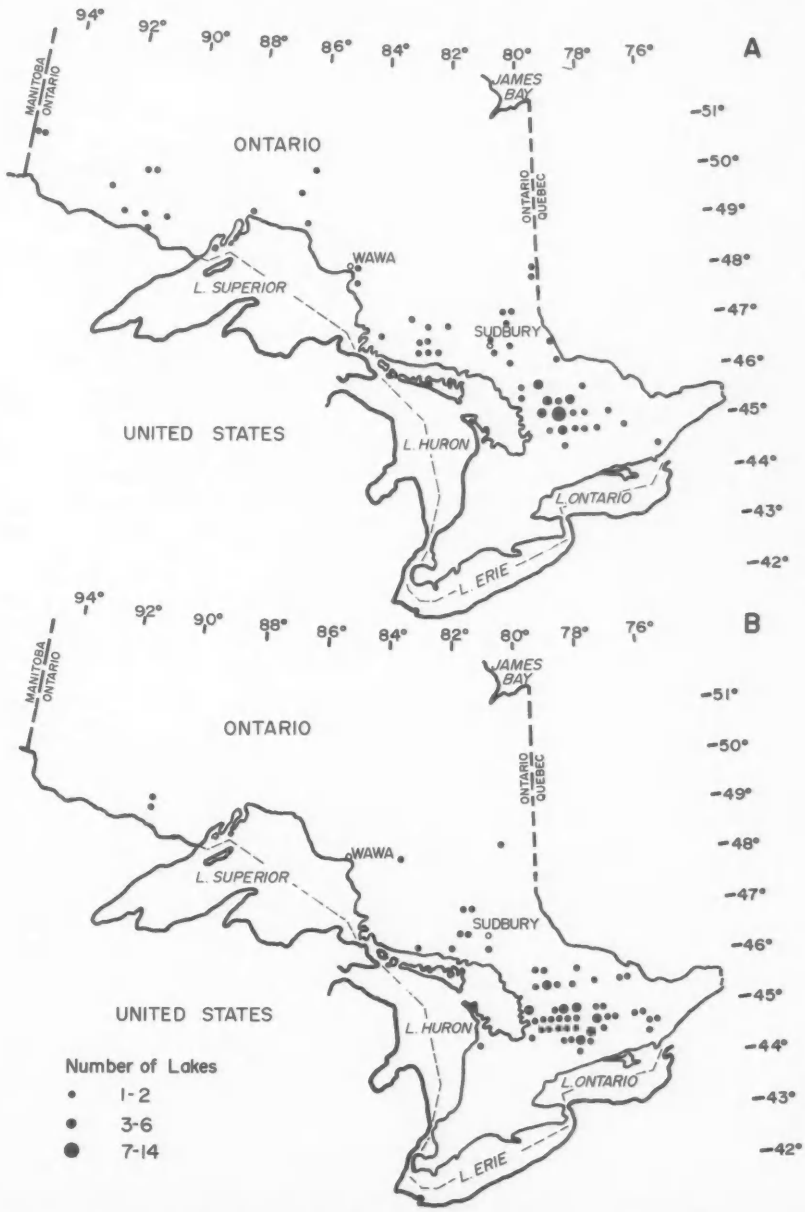

FIG. 1. Distribution of study lakes in Ontario. (A) lake trout (N = 91 lakes), (B) smallmouth bass (N = 90 lakes).

TABLE 1. Sample size, median, and range for numeric variables in lake trout and smallmouth bass data sets.

Variable	Lake trout lakes			Smallmouth bass lakes		
	N	Median	Range	*N*	Median	Range[a]
Predicted Hg ($\mu g \cdot g^{-1}$ wet weight)[b]	91	0.26	0.05–1.16	90	0.34	0.11–0.94
Lake area (ha)	86	225.9	13.7–12 205.8	89	336.8	9.4–12 215.3
Watershed area (ha)	43	2070	171–101 450	53	5047	161–122 475
Watershed area/lake volume (m)	42	0.87	0.12–25.28	53	1.66	0.08–25.03
Maximum depth (m)	85	35.1	6.1–86.9	89	23.2	2.1–86.9
Mean depth (m)	83	11.0	2.1–30.7	88	7.7	1.8–30.7
Elevation (m)	85	363	123–488	88	299	124–476
Secchi depth (m)	83	5.6	1.8–22.0	87	4.3	1.2–9.1
pH	86	6.55	5.07–8.07	72	6.96	5.62–8.58
Alkalinity ($mg \cdot L^{-1}$)[c]	85	3.58	−0.35–94.8	71	6.53	0.01–106.0
Conductivity ($\mu S \cdot cm^{-1}$)	85	35.0	17.7–213.0	67	44.2	26.0–256.0
Colour (apparent) (HU)	55	10.0	0–30.0	15	22.9	7.0–46.0
DOC ($mg \cdot L^{-1}$)[d]	64	3.1	0.3–6.1	55	3.9	2.1–7.4
Ca ($mg \cdot L^{-1}$)	80	3.0	1.8–35.8	60	3.9	2.1–37.3
Mg ($mg \cdot L^{-1}$)	71	0.82	0.32–3.45	59	1.07	0.48–9.68
Na ($mg \cdot L^{-1}$)	69	0.80	0.40–8.78	57	1.35	0.55–5.09
K ($mg \cdot L^{-1}$)	69	0.45	0.24–1.31	57	0.61	0.28–1.43
SO_4 ($mg \cdot L^{-1}$)	75	8.20	0.42–19.2	60	7.7	2.5–12.3
Al ($mg \cdot L^{-1}$)	67	0.022	0.002–0.170	55	0.024	0.001–0.150
Mn ($mg \cdot L^{-1}$)	69	0.016	0.001–0.100	54	0.015	0.001–0.134
Fe ($mg \cdot L^{-1}$)	25	0.04	0.01–0.94	16	0.04	0.01–0.31

[a]Samples with nondetectable concentrations of Al, Mn, and Fe were given values of 0.001, 0.001, and 0.01, respectively. These values are one-half of the detection level cited in OMOE (1981).

[b]Hg concentration predicted for lake trout 44 cm in total length and smallmouth bass 31 cm in total length.

[c]Total inflection point alkalinity.

[d]Dissolved organic carbon.

lakes, fish were sampled in a single year. The year of sampling ranged from 1978 to 1984; sampling effort was evenly distributed across this period for lake trout, and concentrated near the beginning of the period for smallmouth bass. Samples were collected throughout the open-water season with the majority taken during the summer months. Whenever possible, 15–20 fish of representative size were sampled at each site. Total length, weight, and sex were recorded for the fish collected. A boneless, skinless fillet of dorsal muscle tissue was removed from the fish, then packaged and frozen for shipment to Ontario Ministry of the Environment laboratories (OMOE and OMNR 1987). In the laboratory, samples were acid-digested and analyzed for total mercury by cold vapour atomic absorption spectrophotometry (OMOE 1981). Concentrations were reported as micrograms per gram wet weight. Leung and Coceancic (1988) described quality assurance measures taken in the laboratory and estimated the data variation through the entire sampling and analytical process. The lower detection limit was 0.03 $\mu g \cdot g^{-1}$. The mean recovery of sample spikes was 98.3% with a standard deviation of 9.0%. The precision calculated for duplicate samples at 20–50% of the calibration range (0–1.6 $\mu g \cdot g^{-1}$) was 0.020 $\mu g \cdot g^{-1}$.

Lake Selection

Individual lakes were included in our data set if at least 10 smallmouth bass or lake trout had been analyzed for mercury, and a statistically significant regression ($P \leq 0.05$) existed between log mercury concentration and total length. The semi-log relationship between mercury and length was the most linear for both species. Of the fish populations examined, most (113/115 or 98% for lake trout, 90/98 or 92% for smallmouth bass) had a significant linear relationship between log mercury and total length. None of the study lakes were adjacent to a known point source of mercury. Data on 90 lakes with smallmouth bass and 91 lakes with lake trout were retained for further analysis; 14 of the lakes contained both species.

The sampled lake trout lakes were distributed widely across the province, but most were in south-central Ontario (Fig. 1A). Almost all of the lakes were situated on the granitic bedrock of the Precambrian shield and typically were oligotrophic with low alkalinity (Table 1). The smallmouth bass lake set covered a slightly smaller geographic area than the lake trout set (Fig. 1B); again, most lakes were found in south-central Ontario, but some (in the southeastern corner of the distribution) were not located on granitic bedrock and those had higher alkalinity.

There is a gradient of SO_4 deposition in Ontario with lakes in the northwest receiving the least SO_4 deposition and lakes in south-central Ontario receiving the most (Tang et al. 1986). Lakes below the latitude of approximately 46°30′ receive more than 20 kg of wet $SO_4 \cdot ha^{-1} \cdot yr^{-1}$. Atmospheric mercury deposition can be an important source of mercury for lakes (Glass et al. 1986), but the pattern of deposition across Ontario is not known.

Predicting Mercury Concentrations at Standardized Length

The regression of $\log_{10}$ mercury concentration against total length was calculated for each lake trout and smallmouth bass population and used to predict the concentration of mercury at a standardized length. Comparisons could then be made between populations of fish differing in length distribution. Standardized lengths were selected to represent fish that had reached sexual maturity and were still growing. The lengths chosen (44 cm for lake trout, 31 cm for smallmouth bass) were

near the mean lengths of lake trout (47.2 cm) and smallmouth bass (31.3 cm) sampled in the Ontario Contaminants in Sport Fish Program. In no lake was the standardized length outside the length range of the sampled fish.

Predicted mercury concentrations exceeded 0.5 $\mu g \cdot g^{-1}$ in 11% of the lake trout populations and in 22% of the smallmouth bass populations.

Water Chemistry and Morphometric Data

Data on 20 physical and chemical lake descriptors were compiled (Table 1). The principal source of these data was a survey conducted by the Ontario Ministries of the Environment and Natural Resources to assess the sensitivity of Ontario lakes to acidification (Green et al. 1987). Lake morphometry and other physical variables, with the exception of watershed area, were measured as outlined by Dodge et al. (1984). Watershed boundaries were traced after stereoscopic examination of aerial photographs. Area was then calculated with an electronic planimeter.

Water samples for chemical analysis were collected between 1978 and 1986. Each lake was sampled once during this period, usually during the fall, winter, or summer. Most water samples (73% for lake trout and 79% for smallmouth bass) were of the tube composite type, collected at mid-basin and extending to a depth of 5 m, or to 1 m from the bottom in lakes less than 6 m deep. The remaining samples, in order of decreasing frequency, were tube samples taken to 1 m below the thermocline, surface grab samples and volume-weighted composite samples. Analytical methods for water chemistry variables are described in OMOE (1981).

The mean difference between the time of fish sampling and water chemistry sampling was 2.2 yr for lake trout (90% ≤4 yr, maximum = 8 yr) and 2.4 yr for smallmouth bass (83% ≤4 yr, maximum = 8 yr).

Evidence indicates that the pH of some low alkalinity lakes in Ontario has declined over the period of sampling for our study due to acidic deposition, but the rate of decline has probably not been rapid enough to affect our results. For example, the pH of Plastic Lake, in the Muskoka-Haliburton area, decreased by an average of 0.035 pH units per year between 1980 and 1985 (Dillon et al. 1987). Most of our study lakes have a higher alkalinity than Plastic Lake (alkalinity = 0.74 $mg \cdot L^{-1}$ in 1980–81) and would therefore have declined less in pH if they have declined at all.

Data Analysis

We examined the relationship between predicted mercury concentrations and lake variables in three ways: (1) calculation of a Pearson product-moment correlation matrix of all variables, (2) stepwise multiple linear regression of predicted mercury concentration on a reduced set of lake variables, and (3) stepwise regression of predicted mercury concentration on principal components calculated from lake variables. All statistical analyses were done with the Statistical Analysis System (SAS 1982).

Residuals of the individual regressions of $\log_{10}$ predicted mercury concentration on each lake variable were examined and transformations were performed as necessary to achieve homogeneity of variance and normality. The transformations selected differed between variables and between species.

A subset of lake variables was chosen to represent overall physical and chemical characteristics of lakes and to minimize intercorrelations between variables. The subset consisted of lake area, pH, dissolved organic carbon (DOC), calcium, latitude, and elevation. The advantage of regressing mercury on these variables is that the identity of the independent variables is straightforward. The difficulty with it is that intercorrelations between variables can interfere with interpretation of the regression model selected. The model may contain variables not functionally related to the dependent variable (mercury).

The principal components analysis of lake variables avoids the problem of intercorrelations — principal components are by definition uncorrelated linear combinations of the original variables (Dillon and Goldstein 1984) — but at the risk of obtaining variables with an ambiguous identity. All of the lake variables listed in Table 1 were included in the principal components analysis, with the exception of watershed area, the ratio of watershed area to lake volume, colour, and iron, which were dropped due to small sample sizes. The first six principal components were rotated orthogonally (VARIMAX option) to simplify their structure. The rotated principal components were then entered into a stepwise regression with log mercury as the dependent variable.

Lakes with missing variables were excluded from the stepwise regression and principal components analyses. The geographic range of lakes included in the analysis was therefore restricted. For lake trout, all of the lakes northwest of Wawa (see Fig. 1) were excluded, as well as some other lakes dis-

TABLE 2. Lake variables significantly ($P \leq 0.05$) correlated with log mercury concentration in lake trout.

Lake variable	Pearson correlation coefficient	N	P
Log lake area	0.36	86	0.0006
Log watershed area	0.43	43	0.0040
Square root colour	0.53	55	0.0001
DOC	0.60	64	0.0001
Inverse Na	−0.25	69	0.0361
Inverse K	−0.25	69	0.0386
SO_4	−0.24	75	0.0401
Log Al	0.25	67	0.0398
Log Fe	0.53	25	0.0065
Log Secchi	−0.44	83	0.0001

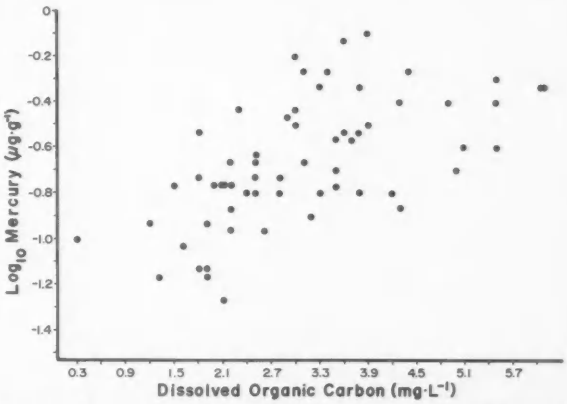

FIG. 2. Mercury concentration (wet weight) in dorsal muscle tissue of lake trout plotted against dissolved organic carbon in Ontario lakes. N = 61 lakes.

TABLE 3. Structure of rotated principal components calculated from the variables describing lake trout lakes. $N = 53$ lakes. Only correlation coefficients with $P \leq 0.01$ are shown.

	Principal component					
Lake variable	1 Hardness	2 Acidity	3 Lake size	4 Latitude/longitude	5 Dystrophy	6 Elevation
Log lake area			0.73			
Maximum depth			0.96			
Mean depth			0.83			
Elevation						0.90
Log Secchi					−0.58	0.68
pH	−0.49	−0.75				
Log alkalinity	−0.57	−0.62			0.43	
Inverse conductivity	0.89					
DOC	−0.44				0.82	
Inverse Ca	0.81	0.42				
Log Mg	−0.82					
Inverse Na	0.71					0.35
Inverse K	0.78					
SO_4	−0.88					
Log Al		0.81				
Log Mn		0.76				
Latitude				0.95		
Longitude				0.96		
Variance explained (%)	28	15	14	11	10	9

TABLE 4. Lake variables significantly ($P \leq 0.05$) correlated with log mercury concentration in smallmouth bass.

Lake variable	Pearson correlation coefficient	N	P
pH	−0.39	72	0.0008
Log alkalinity	−0.39	71	0.0007
Inverse conductivity	0.47	67	0.0001
Inverse Ca	0.46	60	0.0002
Inverse Mg	0.52	59	0.0001
Inverse Na	0.31	57	0.0199
Log K	−0.28	57	0.0341
SO_4	−0.27	60	0.0361
Log mean depth	0.25	88	0.0191

TABLE 5. Stepwise multiple regression of log mercury concentration on log lake area, pH, DOC, inverse calcium, latitude, and elevation for smallmouth bass. $N = 55$ lakes, Model $r^2 = 0.49$, Intercept = 6.274.

Independent variable	Slope	Partial r^2	F	P
Inverse Ca	1.210	0.22	14.55	0.0004
DOC	0.070	0.16	13.16	0.0007
Latitude	−0.160	0.11	11.04	0.0017

tributed across the rest of the study area. For smallmouth bass, lakes at the same latitude as Wawa and north of Wawa were excluded; some lakes at the southern limit of the distribution were also excluded.

Results

Lake Trout

The lake descriptors most highly correlated ($P \leq 0.01$) to log mercury concentration were DOC, square root colour, log iron, log Secchi, log watershed area, and log lake area (Table 2). The first four of these variables, indicative of lake dystrophy, were intercorrelated. Examples of these intercorrelations included DOC and square root colour ($r = 0.62$, $P \leq 0.0001$), DOC and log iron ($r = 0.77$, $P \leq 0.0001$), and DOC and log Secchi ($r = -0.67$, $P \leq 0.0001$). Variables related to lake acidity were not significantly correlated (pH, log alkalinity) or were weakly correlated (SO_4, log aluminum) to log mercury in lake trout.

Of the reduced set of lake variables included in the stepwise regression analysis, only DOC explained a significant ($P \leq 0.05$) amount of variation in log mercury concentration (log Hg = $-1.072 + 0.132$ DOC, $r^2 = 0.37$, $P \leq 0.0001$, $N = 61$ lakes). The positive regression coefficient for DOC (0.132) indicates that higher DOC is associated with higher mercury concentration. The relationship between log mercury and DOC appeared to be strongest at values of DOC less than 4.0 $mg \cdot L^{-1}$ (Fig. 2).

The first six principal components calculated from the lake variables accounted for 87% of the variation in the entire lake variable data set (Table 3). Variables related to water hardness were highly correlated with the first principal component. Of these, inverse conductivity, inverse calcium, inverse sodium and inverse potassium had positive loadings and log magnesium and SO_4 had negative loadings. Variables indicating lake acidity were highly correlated with the second principal component. Principal component 3 represented lake size, as the loadings for log lake area, maximum depth, and mean depth were greatest. The fourth principal component was highly correlated with latitude and longitude and represented geographic location. Principal component 5 was correlated with DOC, representing lake dystrophy, and principal component 6 was highly correlated with elevation.

Principal component 5 (dystrophy) was the only principal component to significantly predict log mercury concentration at $P \leq 0.05$ (log Hg = $-0.639 + 0.150$ principal component 5, $r^2 = 0.33$, $P \leq 0.0001$, $N = 53$ lakes). This result agreed

TABLE 6. Structure of rotated principal components calculated from variables describing smallmouth bass lakes. $N = 47$ lakes. Only correlation coefficients with $P \leq 0.01$ are shown.

	Principal component					
Lake variable	1 Hardness/acidity	2 Lake size	3 Dystrophy	4 Latitude	5 Aluminum	6 Manganese
Log lake area		0.77				
Log maximum depth		0.91				
Log mean depth		0.84				
Elevation			0.66		0.38	
Secchi			0.82			
pH	−0.92					
Log alkalinity	−0.92					
Inverse conductivity	0.96					
DOC			−0.70			
Inverse Ca	0.94					
Inverse Mg	0.95					
Inverse Na	0.57					
Log K	−0.88					
SO_4	−0.61			0.46		
Log Al					0.93	
Log Mn						0.93
Latitude	0.41			0.76		
Longitude	0.69			0.51		
Variance explained (%)	38	14	12	8	8	7

TABLE 7. Stepwise multiple regression of log mercury concentration on rotated principle components for smallmouth bass. $N = 47$ lakes, Model $r^2 = 0.47$, Intercept $= -0.415$.

Independent variable	Slope	Partial r^2	F	P
Principal component 1 (hardness/acidity)	0.098	0.23	13.72	0.0006
Principal component 4 (latitude)	−0.056	0.16	11.94	0.0012
Principal component 3 (dystrophy)	−0.082	0.08	6.15	0.0171

with that for stepwise regression on the reduced set of lake variables.

Smallmouth Bass

Lake variables highly correlated ($P \leq 0.01$) with log mercury in smallmouth bass were inverse magnesium, inverse calcium, inverse conductivity, pH, and log alkalinity (Table 4). These variables, which reflected both water hardness and lake acidity, were highly intercorrelated. Examples of these intercorrelations included pH and log alkalinity ($r = 0.94$, $P \leq 0.0001$), pH and inverse calcium ($r = -0.94$, $P \leq 0.0001$), and inverse calcium and inverse conductivity ($r = 0.96$, $P \leq 0.0001$). Variables indicating lake dystrophy, including DOC, square root colour, and Secchi were not significantly correlated to log mercury, nor were the other variables that were correlated to log mercury in lake trout (log watershed area, log lake area, and log iron).

Stepwise multiple regression identified three variables that were significant ($P \leq 0.05$) in explaining variation in log mercury; these were inverse calcium, DOC, and latitude in order of decreasing importance (Table 5). Inverse calcium had a positive coefficient (slope), indicating that calcium is negatively related to log mercury. Calcium and pH did not appear as significant variables in the same regression, presumably because they were highly correlated. When inverse calcium was withheld from the stepwise regression procedure, the variables selected were pH, DOC, and latitude in that order. DOC was positively related to log mercury when selected after inverse calcium or pH (Table 5), and latitude was negatively related to log mercury. Fish in the northernmost lakes therefore tended to have lower mercury concentrations.

The first six principal components calculated from smallmouth bass lake variables accounted for 86% of the variance in the lake variable data set (Table 6). All of the variables associated with water hardness and acidity were loaded on the first principal component. Principal component 2 represented lake size and principal component 3 represented lake dystrophy. Principal components 4, 5, and 6 were highly correlated with latitude, aluminum, and manganese, respectively.

Principal components 1, 4, and 3 were retained, in that order, in the regression equation with log mercury at $P \leq 0.05$ (Table 7). An inverse relationship between water hardness/pH (principal component 1) and mercury concentration in smallmouth bass was indicated (positive coefficient for principal component 1, negative loadings or inverse transformation for variables in principal component 1). Latitude (principal component 4) was also inversely related to mercury concentration in bass, whereas the relationship between lake dystrophy (principal component 3) and mercury was positive. The regression model composed of these three principal components explained 47% of the variation in log mercury. For smallmouth bass, as for lake trout, the principal components analysis supported the multiple regression analysis on the subset of lake variables.

Discussion

Our results indicate that different environmental factors are associated with mercury accumulation in lake trout and smallmouth bass. DOC was the only variable significant in predicting mercury levels in lake trout. Water hardness and/or acidity best explained mercury levels in smallmouth bass, although DOC and latitude explained significant quantities of residual

variation. Possible explanations for these differences are considered below.

Physical and chemical characteristics of the two lake sets differ, and are probably representative of the natural habitat of lake trout and smallmouth bass in Ontario. For example, levels of DOC tend to be lower in lake trout lakes than in smallmouth bass lakes. Twelve of the 61 lake trout lakes (20%) with DOC data had a DOC below the minimum DOC (2.1 $mg \cdot L^{-1}$) in the smallmouth bass lakes (Table 1). This difference does not appear to explain the lack of correlation between DOC and mercury concentration in smallmouth bass lakes. A strong correlation was found between DOC and mercury concentration in lake trout over the DOC range of 2.1 – 4.0 $mg \cdot L^{-1}$ (Pearson coefficient $= 0.53$, $P = 0.0007$, $N = 37$), but there was no corresponding correlation for smallmouth bass lakes over this range (Pearson coefficient $= 0.007$, $P = 0.97$, $N = 30$).

There is also a slight difference in median alkalinity between the lake trout lakes and the smallmouth bass lakes, although the ranges are similar (Table 1). This is probably because some of the bass lakes are located away from the Precambrian shield. To determine if the difference in alkalinities could have affected our results, we repeated the stepwise regression analysis for smallmouth bass on a subset of 46 lakes located entirely on Precambrian granite (those in the Muskoka-Haliburton area), and obtained results similar to those for the entire smallmouth bass lake set. pH and DOC were selected as significant predictors of log mercury concentration, explaining slightly more of the variation (50%) in mercury for this region than for the complete lake set. Latitude was not a significant variable in the equation, probably because of the restricted geographic range of the lakes. Factors other than differences in the characteristics of our lake sets are probably responsible for the results we obtained for lake trout and smallmouth bass. Differences in the diet of these species is a possible area for further research. The presence of rainbow smelt (*Osmerus mordax*) in the diet appears to affect accumulation of mercury in lake trout and other piscivorous fish (Akielaszek and Haines 1981; MacCrimmon et al. 1983; Mathers and Johansen 1985).

The relationship of DOC to mercury accumulation in fish has received little attention. Bloomfield et al. (1980) and Landner and Larsson (1972) reported elevated mercury levels in fish tissue in highly coloured lakes. Heiskary and Helwig (1986) found a positive correlation between mercury concentrations in northern pike (*Esox lucius*) and the colour of northern Minnesota lakes. A relationship between mercury accumulation in fish and flooding of terrestrial vegetation, peat and humus has also been observed (Bodaly et al. 1984).

The correlation between lake dystrophy and mercury concentration in fish may be related to the role of organic matter in transporting mercury and other metals through aquatic systems. There is a strong affinity between heavy metals and organic matter, particularly humic substances (Förstner and Wittmann 1979). Organo-metallic compounds present in soils, lake sediments, and lake water are carried through the watershed via runoff. Lakes receiving greater amounts of humic matter (as indicated by DOC concentrations) probably also have increased loadings of mercury. A second hypothesis, proposed by Bodaly et al. (1984) to explain elevated levels of mercury in fish following flooding, is that input of organic material stimulates bacterial production of methyl mercury, the form most readily accumulated by fish. Furutani and Rudd (1980) showed that increased rates of methylation result from addition of bacterial substrate to either lake water or sediment-floc samples. To extend this line of reasoning, it is possible that in extremely clear lakes, such as the lake trout lakes in which we found a relationship between DOC in water and mercury in fish, the quantity of organic matter in the water column limits the net rate of bacterial methylation of mercury. In general, however, most of the DOC in lake water is resistant to bacterial decomposition (Wetzel 1975).

The inverse relationship between waterborne calcium and mercury concentration in smallmouth bass in our study may be due either to the correlation of calcium with acidification variables or to an independent effect of calcium on gill permeability. Rodgers and Beamish (1983) showed that the relative efficiency of methyl mercury uptake by rainbow trout (*Salmo gairdneri*) was much lower in hard water than in soft water. The changes in uptake of methyl mercury with water hardness were thought to be due to Ca^{2+} induced changes in gill permeability (Rodgers and Beamish 1983). McFarlane and Franzin (1980) observed that mercury concentrations in livers of northern pike and white sucker (*Catostomus commersoni*) were inversely related to calcium concentration in water from five lakes near Flin Flon, Manitoba.

Numerous authors have reported a relationship between lake pH or alkalinity and mercury accumulation in fish. Landner and Larsson (1972) found a significant negative correlation between methyl mercury content in "pike" muscle and the pH of the water in three small, oligotrophic lakes in western Sweden. Wiener (1983) observed that mercury concentrations in muscle tissue of walleye (*Stizostedion vitreum*) from two naturally acidic lakes were greater than those from three circumneutral lakes in northern Wisconsin. Scheider et al. (1979) compared mercury concentrations of walleye in 52 southern Ontario lakes and found significantly higher mercury values in low alkalinity lakes. Suns et al. (1987) found an inverse correlation ($r = -0.80$, $P < 0.01$) between lake water pH and whole body concentrations of mercury in yearling yellow perch (*Perca flavescens*) in 15 lakes in the Muskoka-Haliburton area of Ontario. They also found a significant negative correlation ($r = -0.91$, $P < 0.01$) between mercury concentrations in the dorsal muscle of a standard-sized (30 cm) smallmouth bass and lake water pH in an overlapping set of 10 lakes. Lake pH was significantly correlated ($r = -0.66$, $P < 0.01$) with mean mercury concentration in pumpkinseeds (*Lepomis gibbosus*) collected from 16 waters in south-central Ontario (Wren and MacCrimmon 1983). Lake pH, alkalinity, and calcium were inversely related to mercury levels in northern pike in northern Minnesota lakes (Heiskary and Helwig 1986). In an experimental study, Wiener (1987) found a small but significant increase in mercury concentration in whole yearling yellow perch following experimental acidification of one basin of a Wisconsin lake by 0.5 pH units.

Acidification of a lake may influence mercury uptake in several ways. A decrease in water column pH allows increased desorption of mercury (and other metals) from suspended particles and thereby increases the availability of mercury for methylation reactions (Wood 1980). Xun et al. (1987) found that a reduction in pH increases net methyl mercury production in both the water column and the oxygenated sediments. Low pH favours the formation of monomethyl mercury over dimethyl mercury (Fagerström and Jernelöv 1972, Wood 1980). Of these two compounds, monomethyl mercury is more readily transferred through the food chain. Shifts in available food organisms due to acidification may also affect mercury accumulation

in fish if the food items substituted have a higher mercury concentration than the original ones (Lindqvist et al. 1984).

The inverse relationship we observed between latitude and mercury in smallmouth bass may be related to the proximity of the more northerly study lakes to the metal smelters in Sudbury (Fig. 1B). Wren and Stokes (1988) found that mercury levels in perch and crayfish increased linearly with distance from Sudbury. They attributed this to elevated concentrations of metals other than mercury which may inhibit methyl mercury production and accumulation in biota. However, there are too few northern lakes in our data set to allow a distinction between the influence of Sudbury-area smelters and of other possible factors related to latitude, such as differences in growth rate of fish or a gradient in mercury deposition.

The relative magnitude of the effects of the factors that we have considered in this study may vary depending on the combination of physical and chemical characteristics of a given lake. We can say, however, that for lake trout lakes in Ontario the most significant variable appears to be the concentration of dissolved organic carbon in the water column. Lake trout from very clear lakes will, in general, have lower mercury levels than those from more coloured lakes. For smallmouth bass lakes, ionic variables, i.e. pH and/or water hardness, are more important. A distinction between the effects of pH and water hardness may not be meaningful for lakes receiving high SO_4 deposition, as softwater lakes will generally also be acidic.

We used available survey data to identify variables associated with mercury accumulation in lake trout and smallmouth bass. However, the amount of variation explained by regression models could be increased if data were collected specifically for this purpose. Information on length at age would reduce variation due to differences in growth rate between lakes. Measures of lake productivity, mercury deposition, and mercury present in sediments might also improve our ability to predict mercury concentrations in fish.

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Roots versus Shoots in Nutrient Uptake by Aquatic Macrophytes in Flowing Waters

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Transplant experiments conducted in the South Saskatchewan River, Saskatchewan, Canada showed that the aquatic macrophyte, *Potamogeton crispus*, obtained most of its nutrients through the roots. When plants were grown in buckets containing high (602 $\mu g \cdot g^{-1}$ total phosphorus; 712 $\mu g \cdot g^{-1}$ total organic nitrogen) or low (258 $\mu g \cdot g^{-1}$ total phosphorus; 109 $\mu g \cdot g^{-1}$ total organic nitrogen) nutrient sediments and exposed to high (75 $\mu g \cdot L^{-1}$ soluble reactive phosphorus (SRP); 530 $\mu g \cdot L^{-1}$ total dissolved nitrogen (TDN), or low (10 $\mu g \cdot L^{-1}$ SRP; 323 $\mu g \cdot L^{-1}$ TDN) nutrient concentrations in the open water, biomass, shoot density and tissue nutrient concentrations were largely determined by sediment type, rather than open water chemistry. These results indicate that the roots are an important site of nutrient uptake for aquatic macrophytes in flowing waters.

Les expériences de transplantation faites dans la rivière South Saskatchewan en Saskatchewan, Canada, ont montré que la plante macrophyte aquatique, *Potamogeton crispus*, se procurait la plupart des substances nutritives dont elle a besoin grâce à ses racines. Lorsque des plantes étaient laissées à pousser dans des seaux contenant des sédiments riches (602 $\mu g \cdot g^{-1}$ en phosphore total; 712 $\mu g \cdot g^{-1}$ en azote organique total) ou pauvres (258 $\mu g \cdot g^{-1}$ en phosphore total; 109 $\mu g \cdot g^{-1}$ en azote organique total) en substances nutritives et qu'elles étaient exposées à des concentrations en substances nutritives élevées (75 $\mu g \cdot L^{-1}$ de phosphore réactif soluble, 530 $\mu g \cdot L^{-1}$ d'azote dissous total) ou peu élevées (10 $\mu g \cdot L^{-1}$ de phosphore réactif soluble, 323 $\mu g \cdot L^{-1}$ d'azote dissous total) dans l'eau non liée, on a constaté que la biomasse, la densité des pousses et la concentration en substances nutritives des tissus étaient largement déterminées par le type de sédiments plutôt que par la chimie de l'eau non liée. Ces résultats indiquent que les racines constituent un site important d'absorption des substances nutritives chez les plantes macrophytes aquatiques en eau courante.

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The role of sediments versus open water in supplying nutrients to aquatic macrophytes has long been debated, particularly with respect to the management of aquatic weeds and the control of lake eutrophication (Hutchinson 1975; Best and Mantai 1978; Carignan and Kalff 1980; Agami and Waisel 1986). Several studies have clearly demonstrated that the sediments are the major source of phosphorus and nitrogen for rooted aquatic plants growing in lakes (Nichols and Keeney 1976a,b; Best and Mantai 1978; Carignan and Kalff 1980; Barko and Smart 1980, 1981; Smith and Adams 1986). However, for aquatic macrophytes growing in flowing waters, the nutrient source has yet to be quantified. The observation that the bottom sediments of many rivers are too coarse to represent a significant nutrient pool and that flowing water provides a continuous supply of nutrients and prevents localized patches of nutrient depletion around the shoot has led to the assumption that aquatic macrophytes in rivers obtain most of their nutrients from the open water. Nevertheless, studies on flowing waters have failed to demonstrate any correlation between open-water nutrient concentrations and aquatic macrophyte biomass or tissue nutrient concentrations (Owens and Edwards 1961; Ladle and Casey 1971; Kern-Hanson and Dawson 1981; Blachford and Ongley 1984). This inconsistency has been attributed to the presence of growth-limiting factors other than nutrients (Kern-Hanson and Dawson 1981) or open-water nutrients in excess of the plants' requirements (Owens and Edwards 1961; Blachford and Ongley 1984). However, the possibility remains that aquatic weeds in flowing waters are obtaining their nutrients from the bottom sediments.

The aim of our study was to determine the role of roots versus shoots in nutrient uptake by aquatic angiosperms in flowing waters. In many rivers on the North American prairies, increased nutrient loading (due to sewage, agricultural and/or industrial inputs) and water withdrawal (for irrigation, munic-

ipal, and industrial requirements) have been associated with an abundant growth of aquatic plants (Tones et al. 1980; Blachford and Ongley 1984; Charlton et al. 1986). As a first step towards establishing cost-efficient guidelines for nutrient loading so as to reduce aquatic macrophyte growth, the source of nutrients utilized by aquatic plants in rivers must be established. The approach we adopted was a transplant experiment, conducted in the South Saskatchewan River, Saskatchewan, Canada. *Potamogeton crispus* L. was grown in pails containing natural sediments with different levels of nitrogen and phosphorus, at two sites with very different concentrations of nitrogen and phosphorus in the open water. The effects of variations in sediment to water phosphorus and nitrogen ratios could therefore be examined in relation to plant growth and nutrient uptake.

Materials and Methods

Study Site

The South Saskatchewan River is a seventh-order stream and a major tributary of the Saskatchewan–Nelson river system. It originates in south-eastern Alberta, Canada with the convergence of the Bow and Oldman rivers and flows northeast across Saskatchewan, joining with the North Saskatchewan River east of Prince Albert, Saskatchewan to form the Saskatchewan River. The latter flows into Cedar Lake and then Lake Winnipeg, eventually draining into Hudson Bay through the Nelson River. Throughout much of the South Saskatchewan River, flows are regulated by the Gardiner Dam located 114 km upstream of Saskatoon. Operating guidelines for the dam require that a minimum flow of 42.5 $m^3 \cdot s^{-1}$ be maintained in the river at Saskatoon. During the past 10 yr, mean monthly flows (May – September) at Saskatoon averaged 121 $m^3 \cdot s^{-1}$.

Sewage from the City of Saskatoon has been discharged into the South Saskatchewan River for over 75 yr and represents the major source of nutrients to the river (Tones et al. 1980). Water quality assessments during the past 20 yr have documented increased nitrogen and phosphorus concentrations, and increased macrophytic and periphytic growth downstream of the Saskatoon sewage treatment plant outfall (Tones et al. 1980; Kerc 1986). As much as 80% of the total phosphorus (TP) and 50% of the total Kjeldahl nitrogen (TKN) loads in the open water downstream of Saskatoon are due to the sewage effluent, with summer (July–September) TP and TKN loads from the sewage treatment plant averaging 416.7 and 2062.5 $kg \cdot d^{-1}$, respectively, over 2 yr (1984–85) (Kerc 1986). By comparison, TP and TKN loads in the open water upstream of the sewage treatment plant averaged 96.9 and 1595.1 $kg \cdot d^{-1}$ over the same period.

Our study site was located 22 km downstream of the sewage treatment plant. Here, open-water nutrient concentrations and aquatic macrophyte growth along the East riverbank were considerably less than along the West bank as a result of limited mixing between the plume of wastewater effluent from the sewage treatment plant (located on the West bank) and the river water.

Experimental Design

Potamogeton crispus was collected from the South Saskatchewan River on June 28, 1987. Intact rhizomes bearing two to three stems (each <8 cm long) were planted in 2-L plastic pails containing homogenized river sediments which had been collected with a shovel from either 15 km upstream or 22 km downstream (West bank) of the Saskatoon sewage treatment plant. The sediments were packed firmly into the pails such that nutrient exchange between the sediment-bound or porewater pools and the open water was likely minimal. The experimental treatments followed a factorial design whereby replicate buckets (six) of each sediment type (high or low) were placed at a depth of 0.6 m in the river at sites with high or low nutrient concentrations in the open water (i.e. the West and East banks, 22 km downstream of the Saskatoon sewage treatment plant). After 7 wk, plants were harvested, separated into shoot, root, and rhizome, and dried at 110°C to constant weight. Shoot density was determined as the number of stems per pail. Total phosphorus and total organic nitrogen in plant shoots and sediments (collected prior to planting) were extracted by wet oxidation with sulphuric acid and hydrogen peroxide (Parkinson and Allen 1975) and analyzed spectrophotometrically by the phenol–hypochlorite (Haslemore and Roughan 1976) or molyb-

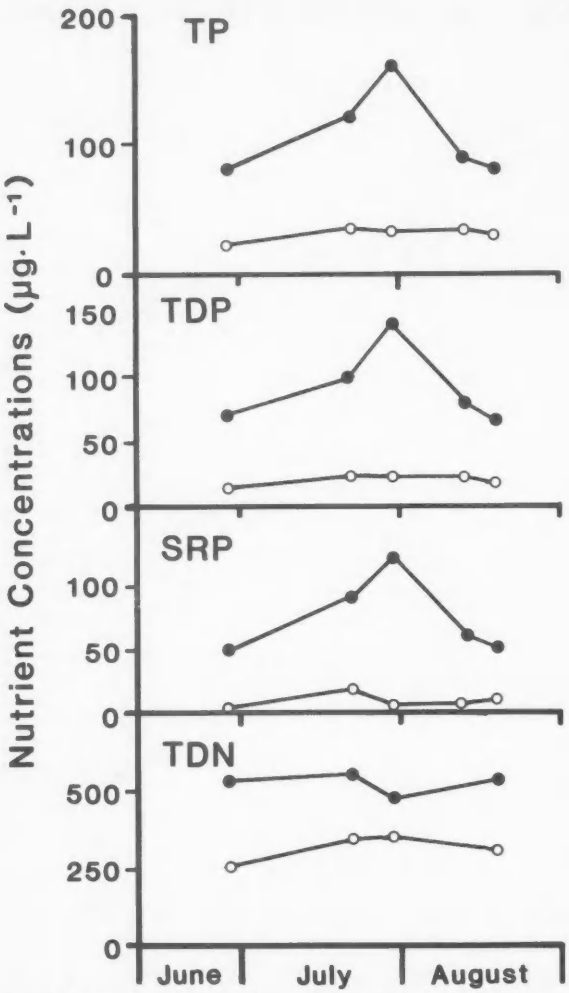

FIG. 1. Open-water total phosphorus (TP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), and total dissolved nitrogen (TDN) concentrations in the South Saskatchewan River, 22 km downstream of the Saskatoon sewage treatment plant, during the transplant experiment: (●) West bank; (○) East bank.

TABLE 1. Physical and chemical parameters of the open water along the West and East banks of the South Saskatchewan River, collected 22 km downstream of the Saskatoon sewage treatment plant on August 19, 1987.

	West bank	East bank
pH	8.26	8.62
Conductivity ($\mu S \cdot cm^{-1}$)	445	425
Alkalinity (mg $CaCo_3 \cdot L^{-1}$)	150	146
Hardness (total as $CaCO_3$)	176.7	172.8
Total dissolved solids ($mg \cdot L^{-1}$)	253	245
Ions ($\mu g \cdot L^{-1}$)		
Bicarbonate	182.9	165.4
Calcium	43.4	42.0
Chloride	8.8	6.9
Magnesium	16.6	16.5
Potassium	2.73	2.49
Sodium	23.6	22.5
Sulphate	67.9	66.7

denum blue (Stainton et al. 1977) colorometric methods, respectively. Exchangeable phosphorus was extracted from fresh sediment by shaking for 16 h with 0.1*N* NaOH–NaCl and measured spectrophotometrically (Stainton et al. 1977). Sediment organic content was measured as loss after ignition at 550°C for 5 h. Sediment water content was measured as loss after drying at 110°C to constant weight. All sediment and tissue analyses were performed in triplicate.

Surface water temperature was measured and surface water samples were collected for phosphorus (total (TP), total dissolved (TDP), and soluble reactive (SRP) phosphorus) and total dissolved nitrogen (TDN) along both river banks every 2–3 wk throughout the experiment. On one occasion (August 19), water samples were also analyzed for pH, conductivity, hardness, alkalinity, total dissolved solids, and dominant ion concentrations. All water chemistry was performed by the Western Regional Water Quality Branch Laboratory, Environment Canada, Saskatoon, using procedures outlined in their manual (Environment Canada 1979). Current velocity and underwater penetration of photosynthetically active radiation (PAR) were measured at each site on three occasions with a Gurly-Price model 22 current meter or a LiCor quantum radiometer, respectively. The vertical attenuation coefficient for PAR (K) was calculated by linear regression from the Beer-Lambert equation.

Data were analyzed with the Statistical Analysis System (SAS Institute Inc. 1982). The response of plant biomass, shoot density, and tissue nutrient levels to variations in sediment type and water quality were determined by analysis of variance, followed by least significant difference multiple comparison of means tests. To stabilize variance, a logarithmic transformation was applied to the biomass and shoot density data.

Results

Environmental Conditions

Phosphorus and nitrogen concentrations in the open water were consistently greater along the West than the East bank for all sampling dates (Fig. 1). Phosphorus concentrations along the West bank increased in late July. Total dissolved nitrogen concentrations, however, remained relatively constant throughout the study. Conductivity, alkalinity, hardness, total dissolved solids, and dominant ion concentrations in the open water were also greater along the West than the East bank (Table 1). These differences were, however, slight as compared with differences in nitrogen and phosphorus levels between the two banks.

Comparison of initial nutrient concentrations, organic matter, and water content in the South Saskatchewan River sediments showed that sediments from downstream of Saskatoon were more fertile than those from upstream (Table 2). The upstream sediments also appeared coarse and sandy as compared with the finer-particle sediments from downstream. The range in sediment phosphorus concentrations we observed was similar to that determined for natural sediments collected from other Canadian prairie river systems (P. A. Chambers, unpubl. data; Cross et al. 1986). Our upstream sediments will henceforth be referred to as low nutrient sediments as compared with the high nutrient downstream sediments.

Attenuation of underwater PAR showed little variation between sampling dates. There was, however, a significant but weak ($0.01 < P < 0.05$) difference between the two riverbanks, with attenuation coefficients of 0.79 ± 0.05 and 1.04 ± 0.06 m^{-1} (mean $\pm$ 1 SE) for the East and West banks, respectively. As the natural plant community colonized the entire riverbed (2 m maximum depth), it is unlikely that plant growth was light-limited at our 0.6 m deep study sites.

Temperature ranged from 16 to 26°C over the study period and did not differ between the East and West banks. Current velocity, measured 0.1 m below the water surface, was 22.9 $cm \cdot s^{-1}$ along both banks on June 28 and decreased to 11.9 $cm \cdot s^{-1}$ by August 19, presumably due to the increased biomass of aquatic weeds which blocked current flow.

Plant Growth

Biomass (total, shoot, root and rhizome) and shoot density of *P. crispus* were consistently greater ($P < 0.01$) in high than in low nutrient sediments (Table 3). Analysis of variance showed that while plant biomass and density were highly dependent upon sediment type ($P < 0.001$), there was no significant response to water phosphorus and nitrogen levels ($P > 0.25$). There was also no significant interaction between open-water and sediment nutrient treatments ($P > 0.75$). On an areal basis, shoot biomass in the low nutrient sediments was approximately 19 g dry weight $\cdot m^{-2}$ compared with 86 g dry weight $\cdot m^{-2}$ in the high nutrient sediments.

Tissue nitrogen and phosphorus concentrations for *P. crispus* were also consistently greater ($P < 0.0001$) in the high than in the low nutrient sediments (Table 4). However, unlike biomass and shoot density which were independent of water quality, tissue phosphorus levels and nitrogen:phosphorus (N/P) ratios showed a significant, but weak, response to water quality. Thus, in low nutrient sediments, tissue phosphorus concentrations were greater ($0.01 < P < 0.05$) and N/P ratios were lower ($0.0001 < P < 0.001$) for plants exposed to nutrient-enriched river water. There was no significant difference ($P > 0.05$) in tissue phosphorus concentrations or N/P ratios for plants growing in high nutrient sediments in either high or low open-water nutrient concentrations.

Discussion

Biomass and shoot density of *P. crispus* were primarily determined by sediment type and not significantly affected ($P > 0.25$) by open-water nutrient concentrations. Thus, plants growing in low nutrient sediments had consistently less biomass

TABLE 2. Chemistry of sediments, collected from the South Saskatchewan River upstream and downstream of Saskatoon, at the start of the transplant experiment (mean ± 1 SE).

	Upstream	Downstream
Total phosphorus ($\mu g \cdot g^{-1}$ dry weight)	258 ± 26	602 ± 9.0
Exchangeable phosphorus ($\mu g \cdot g^{-1}$ dry weight)	11.3 ± 1.0	89.0 ± 3.7
Total organic nitrogen ($\mu g \cdot g^{-1}$ dry weight)	109 ± 4.0	712 ± 9.0
Percent water content	20.3 ± 0.5	30.4 ± 0.1
Percent organic matter	0.48 ± 0.07	2.40 ± 0.04

TABLE 3. Biomass (g dry weight·pail^{-1}) and shoot density of *P. crispus* grown in high or low nutrient sediments at sites with high or low concentrations of nitrogen and phosphorus in the open water. Data were back-transformed after $\log(X+1)$ transformation. For any parameter, values sharing the same superscript are not significantly different ($P > 0.05$; $n = 6$).

Open-water nutrients	Sediment nutrients	Biomass				Shoot density
		Total	Shoot	Root	Rhizome	
Low	High	1.67[a]	1.20[a]	0.27[a]	0.18[a]	60[a]
	Low	0.29[b]	0.19[b]	0.05[b]	0.05[b]	13[b]
High	High	1.83[a]	1.38[a]	0.20[a]	0.17[a]	47[a]
	Low	0.38[b]	0.25[b]	0.04[b]	0.03[b]	12[b]

TABLE 4. Tissue phosphorus (P) and nitrogen (N) concentrations ($mg \cdot g^{-1}$ dry weight; mean ± 1 SE; $n = 6$) for *P. crispus* grown in high or low nutrient sediments at sites with high or low concentrations of nitrogen and phosphorus in the open water. For any parameter, values sharing the same superscript are not significantly different ($P > 0.05$).

Open-water nutrients	Sediment nutrients	Tissue Nutrient Concentrations		
		P	N	N/P
Low	High	3.77 ± 0.11[a]	36.05 ± 1.55[b]	9.57 ± 0.32[a]
	Low	1.82 ± 0.12	24.74 ± 1.20[a]	13.68 ± 0.40
HIgh	High	3.88 ± 0.20[a]	31.66 ± 1.30[b]	8.27 ± 0.58[a]
	Low	2.50 ± 0.21	26.81 ± 1.06[a]	10.87 ± 0.48

and fewer shoots than ones growing in high nutrient sediments. Tissue nutrient concentrations were also largely determined by sediment type. However, tissue phosphorus levels and N/P ratios also showed a significant, but weak, correlation with open-water nutrient concentrations, such that plants growing in low nutrient sediments had higher tissue phosphorus levels and lower N/P ratios when exposed to phosphorus-rich river water.

Our results indicate that nutrient uptake by aquatic macrophytes in the South Saskatchewan River is largely effected by the roots. Studies on European streams and rivers have shown that aquatic macrophyte biomass and tissue nutrient concentrations are not correlated with open-water nutrient concentrations (Owens and Edwards 1961; Ladle and Casey 1971), which is consistent with our observation on the importance of sediment nutrients to the growth of rooted aquatic plants in flowing waters. However, Kern-Hanson and Dawson (1981) observed that for 19 streams in Denmark, aquatic macrophyte biomass and tissue nutrient concentrations were not correlated with either open-water or sediment nutrient concentrations, suggesting that in these streams factors other than nutrients limited growth.

The importance of sediment type in the control of riverine plant growth is consistent with results of nutrient uptake studies on aquatic macrophytes in lakes. Carignan and Kalff (1980) demonstrated that over 70% of total plant phosphorus is derived from the sediments for aquatic angiosperms grown in nonflowing waters with TP concentrations in the open water ranging from 10 to 290 $\mu g \cdot L^{-1}$. Other investigators (Best and Mantai 1978; Barko and Smart 1980; Smith and Adams 1986) have also observed that phosphorus uptake by roots exceeds that by shoots in nature. While the roots of submersed aquatic plants are also capable of nitrogen uptake (Nichols and Keeney 1976a,b; Best and Mantai 1978), the relative contribution of shoots versus roots to nitrogen uptake for aquatic macrophytes in lakes is more equivocal (Agami and Waisel 1986).

In summary, our results showed that *P. crispus*, one of the dominant aquatic macrophytes in the South Saskatchewan River, obtained most of its nutrients through the roots. While further experiments are needed to extend this conclusion to other flowing waters, the generality of our results is supported by our experimental design in which only sediment and open-water nutrient levels varied and other factors likely to affect plant growth (i.e. irradiance, current velocity, temperature, river water–porewater exchange) remained constant. At present, however, the results do not allow any conclusion as to whether the nutrients taken up by the roots are derived from a sediment-bound pool or river water as it passes through coarse-grain sediments. Since increased current velocity results in river beds with larger sediment particles which are usually less nutrient rich and less densely packed, the proportion of river water to sediment-bound nutrients in the rooting zone will depend upon flow rate. Our finding on the importance of root uptake for riverine macrophyte nutrition has important implications for aquatic weed control, suggesting that the impact of decreased nutrient loading on aquatic macrophyte growth will depend upon nutrient availability and particle size of the river bed sediments.

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Bone Characteristics and Metal Concentrations in White Suckers (*Catostomus commersoni*) from One Neutral and Three Acidified Lakes in Maine

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The bone characteristics of white suckers, *Catostomus commersoni*, from four lakes in Maine were studied in relation to lake water quality and metal concentrations in fish. Green Lake had a neutral pH, high buffering capacity, and low aluminum concentrations, whereas the other three lakes had low pH, low buffering capacity, and elevated aluminum concentrations. The concentrations of aluminum in white suckers did not differ among the four lakes, but concentrations of cadmium, lead, and mercury were greater in fish from the three low-pH lakes. The vertebrae were weaker and more flexible in fish from the low-pH lakes than in those from neutral-pH Green Lake. The inferior mechanical properties of bone in fish from the low-pH lakes were probably a result of the significantly lower proline concentrations in collagen. Low pH of lake water or elevated whole-body concentrations of cadmium, lead, and mercury, either individually or combined, could have caused the inferior mechanical properties of bone of white suckers from the low-pH lakes.

Les caractérisques osseuses du meunier noir, *Catostomus commersoni*, de quatre lacs du Maine ont été étudiées en fonction de la qualité de l'eau et de la concentration des métaux dans les tissus. Le lac Green a un pH neutre, une forte capacité-tampon et une faible concentration en aluminium; les trois autres lacs ont un pH bas, une faible capacité-tampon et une forte concentration en aluminium. La concentration tissulaire en aluminium était la même chez les sujets des quatre lacs; chez les sujets des trois lacs à bas pH cependant, le cadmium, le plomb et le mercure étaient trouvés en concentration inférieure à celle des poissons du lac Green. Les vertèbres étaient moins résistantes et plus flexibles chez les sujets des trois lacs. Les propriétés mécaniques inférieures des os des poissons provenant des trois lacs à bas pH sont probablement le résultat d'une concentration significativement plus faible de proline dans le collagène. Le bas pH de l'eau des lacs ou la concentration élevée dans l'ensemble de l'organisme du cadmium, du plomb et du mercure, individuellement ou en synergie, expliquent peut-être pourquoi les os des meuniers noirs dans les lacs à bas pH ont des propriétés mécaniques inférieures.

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Precipitation is much more acidic in the northeastern United States than in western North America or in remote locations elsewhere in the world. Acidification of surface waters has caused complex changes in aquatic communities, the most noticeable being the decline or loss of fish populations (Dillon et al. 1984). Linkages between surface water acidification and fish population decline have been clearly established in Scandinavia (Wright and Henriksen 1983; Muniz et al. 1984), the Sudbury region of Ontario (Harvey and Lee 1982), and southwestern Nova Scotia (Watt et al. 1983). Haines and Baker (1986), who examined the extensive fishery database for the Adirondack Mountain region of New York, concluded that at least 50 fish populations have been eliminated by acidification. Surveys of 22 lakes in Maine showed that the distribution and abundance of fish species were adversely affected by acidity (Haines et al. 1986).

In the present study we evaluated the relation of lake acidity to metal concentrations and bone characteristics in white suckers (*Catostomus commersoni*) from four Maine lakes studied by Haines et al. (1986). Skeletal deformities have been reported in white suckers exposed to low pH in the laboratory (Beamish 1972; Trojnar 1977) and in acidified lakes in Ontario (Beamish et al. 1975; Fraser and Harvey 1982). It was suggested that these deformities resulted from demineralization of the skeleton to maintain calcium balance for osmoregulation and reproduction. However, there have been conflicting reports about whether bone is demineralized in fish exposed to low pH (Lockhart and Lutz 1977; Fraser and Harvey 1982).

We evaluated bone quality in white suckers as a potential biological indicator of sublethal stress from exposure to elevated concentrations of acidity and metals in lakes. Bone characteristics were assessed by determining the composition (bio-

chemical composition), quantity (density), and structural integrity (mechanical properties) of bone. Previous studies of fish exposed to environmental contaminants in laboratory studies have shown that bone characteristics are more sensitive than either survival or growth as indicators of contaminant stress (Hamilton et al. 1981a; Cleveland and Hamilton 1983; Hamilton et al. 1986). Assessment of bone characteristics has also been used to evaluate the impact of contaminants on feral fish populations (Mehrle et al. 1982; Baumann and Hamilton 1984; Buckley et al. 1985).

Methods

Water and Fish Collection

We studied four Maine lakes (Green Lake, Unnamed Pond, Horseshoe Pond, and Lost Pond) that were in forested watersheds without human habitation, had no history of fish stocking or chemical reclamation, and were of similar small size and hydrologic type. The lakes spanned a wide range of pH, and all contained white suckers. Each lake was sampled for water chemistry three times: spring overturn, summer stratification, and fall overturn. The water sampling protocol, which followed that of the National Acid Precipitation Assessment Program (1985), was briefly summarized by Haines et al. (1986).

Fish were collected once from each lake during the summer of 1982 or 1983, in an overnight set of one 60- or 75-m nylon experimental-mesh gillnet. Fish were placed on ice and returned to the laboratory, where they were stored at −23°C before analyses of bone characteristics and metals.

Bone Analyses

We examined 11 – 15 white suckers of similar size from each lake for bone characteristics. Fish from various locations were selected for bone characterization so that the total length of selected animals was within the range 290 – 360 mm. This range encompassed the majority of fish available for selection for testing and met the criterion of less than 10% coefficient of variation in total length of fish between groups for comparing the mechanical properties of bone from fish of different treatments or locations (Hamilton et al. 1981a). Fish were measured for total length and weight, and X-rayed before dissection. Radiographs were examined for abnormalities in vertebral structures. Vertebrae three through 10, counted from the skull, were dissected from each fish, and spines and soft tissue were removed leaving only the centrum. Individual vertebrae were measured with an ocular micrometer and microscope to determine anterior–posterior length and mean radius of vertebrae. These measurements were used to calculate the cross-sectional area and volume on which compressional forces act during loading. Vertebrae were kept moistened with distilled water during dissection and measurement, and stored moistened at 4°C for 2 – 3 h prior to testing. Each vertebra was brought to room temperature over a 15-min period then compression-tested in an anterior–posterior direction with an Instron model 1132 testing machine under a constant strain rate of 5.00mm·min^{-1} to determine strength, elasticity, and energy-absorbing capacity which was termed toughness. We used a typical stress-strain curve resulting from vertebral resistance to a constant-strain compressive load to calculate the mechanical properties; see Hamilton et al. (1981a) for a more detailed explanation of the interpretation of mechanical properties from stress-strain

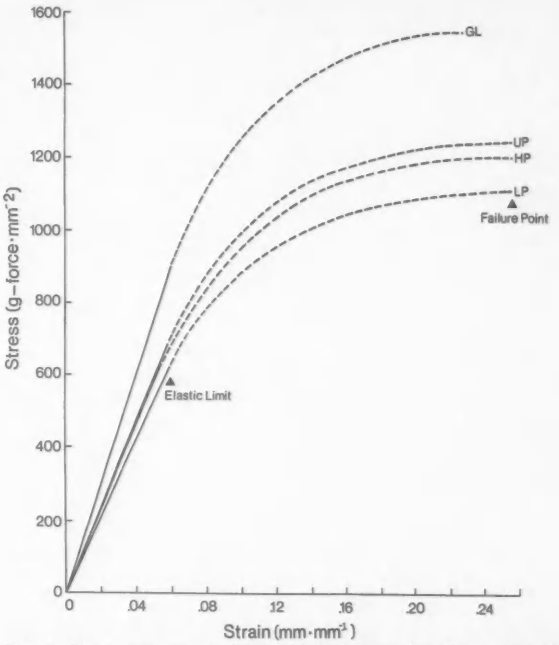

FIG. 1. Stress-strain curves for mechanical properties of vertebrae of white suckers from four Maine lakes; Green Lake (GL), Unnamed Pond (UP), Horseshoe Pond (HP), and Lost Pond (LP). The straight, solid line portion of each curve is the elastic region of the stress-strain curve, whereas the curved, broken line is the plastic region. Elastic limit is the force level above which permanent structural damage occurs in a test vertebra. Failure point is the force level causing vertebral failure.

curves. The elastic region of the stress-strain curve is where a test specimen shows a linear relationship between stress and strain; this portion of a typical stress-strain curve is depicted as a straight, solid line (see Fig. 1). The plastic region of the stress-strain curve is where a test specimen no longer shows a constant stress-strain proportionality; this portion of a typical stress-strain curve is depicted as a curved, broken line (see Fig. 1). Measures of bone strength were elastic limit (the force level above which permanent structural damage occurs) and rupture (the force level causing failure of structural integrity). Elastic limit, i.e. stress at elastic limit, was measured as the force level at the elastic limit of the stress-strain curve and corresponded to the upper limit of the elastic region of the curve. Rupture, i.e. ultimate stress, was measured as the force level at the failure point. Elasticity measures were strain (the amount of deformation incurred by bone tissue at failure) and modulus of elasticity (the index of bone stiffness or elasticity). Strain, i.e. ultimate strain, was measured as the amount of deformation incurred by bone at the failure point, and modulus of elasticity as the slope of the elastic region of the stress-strain curve. Toughness was measured as the total area under the stress-strain curve before the failure point and corresponded to the total work required to break the bone.

After mechanical testing, we measured the concentrations in bone of collagen (Flanagan and Nichols 1962), hydroxyproline (Woessner 1961), proline (Troll and Lindsley 1955), calcium (Gitelman 1967), and phosphorus (Fiske and Subbarow 1925). Density of vertebrae was calculated as mean dry weight (mil-

TABLE 1. Mean (± SE) of chemical variables for the four Maine lakes from which white suckers were collected for analysis of bone characteristics and metal concentrations. Means for a given measure that do not contain the same letter superscript are significantly different ($\alpha < 0.05$, n is given in parentheses). F-ratio degrees of freedom 3, 38–91.

Variable and unit	Lake				F-ratio
	Green	Unnamed	Horseshoe	Lost	
pH	7.02 ± 0.09^{a}(18)	5.83 ± 0.11^{b}(14)	5.61 ± 0.02^{bc}(16)	5.43 ± 0.05^{c}(22)	85.24
Alkalinity ($\mu eq \cdot L^{-1}$)	110 ± 3^{a}(18)	22 ± 2^{b}(14)	6 ± 1^{c}(16)	6 ± 1^{c}(22)	682.86
Conductance ($\mu S \cdot cm^{-3}$)	24 ± 0^{a}(18)	17 ± 1^{b}(14)	17 ± 0^{b}(16)	18 ± 1^{b}(22)	38.99
Color (Pt–Co unit)	10 ± 0^{a}(18)	63 ± 4^{b}(14)	26 ± 2^{c}(20)	34 ± 1^{d}(22)	108.81
Calcium ($\mu eq \cdot L^{-1}$)	88 ± 2^{a}(10)	72 ± 4^{b}(8)	69 ± 1^{b}(12)	66 ± 2^{b}(12)	16.93
Magnesium ($\mu eq \cdot L^{-1}$)	30 ± 1^{a}(10)	28 ± 2^{a}(9)	28 ± 1^{a}(12)	25 ± 1^{b}(12)	6.81
Aluminum ($\mu g \cdot L^{-1}$)	28 ± 3^{a}(22)	66 ± 5^{b}(18)	51 ± 2^{b}(29)	163 ± 11^{c}(26)	78.93

ligrams) per mean volume (cubic centimetres) of the eight vertebrae. Mean dry weight was the total weight of the vertebrae divided by the number of vertebrae in the pooled weight, and mean volume was the average of measurements for individual vertebrae.

Differences in biochemical composition of bone of fish among lakes were evaluated with one-way analysis of variance (ANOVA), whereas differences in density and mechanical properties were evaluated with one-way analysis of covariance in which fish length was used as the covariable. When ANOVA was significant ($P \leq 0.05$), means were compared by the least significant difference multiple-mean comparison test ($\alpha = 0.05$) (Snedecor and Cochran 1967). Simple linear regression was used to evaluate the relation among bone characteristics, metal concentrations in fish, and lake water chemistry.

Metal Analyses

The first five white suckers of each group used in bone analyses were analyzed individually for aluminum, cadmium, lead, mercury, and zinc. In addition to removing eight vertebrae from each fish for analysis of bone characteristics, we removed the gastrointestinal tract to reduce variation in some metals, especially aluminum, caused by unincorporated gut contents (Brumbaugh and Kane 1985). The rest of each fish was chopped into small sections with a large stainless steel knife, and blended for 20 min in a polypropylene container fitted with a Teflon blade assembly. All equipment used in the homogenization procedure was washed in the following sequence between samples: detergent and tap water, 10% HCl, ultrapure water, and distilled acetone. About 100 g of homogenate was lyophilized and dry-blended for 10 min. Samples were stored in polyethylene bags in a desiccator until analyzed. Duplicate 0.5 g subsamples of dry homogenate were digested in 3 mL of 16 M HNO_3 in sealed pressure reaction tubes heated to 70°C for 48 h. Digestates were diluted to 50 mL with 1% HCl.

Aluminum, cadmium, and lead were analyzed by graphite furnace atomic absorption spectrophotometry (AAS); aluminum by four-point standard additions, and cadmium and lead by direct calibration with aqueous standards. Mercury was

TABLE 2. Mean (± SE) of bone characteristics and fish size of white suckers from four Maine lakes. Means for a given measure that do not contain the same letter superscript are significantly different ($\alpha < 0.05$). F-ratio degrees of freedom 3, 46–48.

Characteristic	Lake and pH				F-ratio
	Green 7.0	Unnamed 5.9	Horseshoe 5.6	Lost 5.4	
Mechanical properties					
Rupture ($g\text{-force} \cdot mm^{-2}$)	1556 ± 47^{a}	1248 ± 48^{b}	1209 ± 62^{b}	1116 ± 42^{c}	21.46
Elastic limit ($g\text{-force} \cdot mm^{-2}$)	901 ± 32^{a}	686 ± 33^{b}	633 ± 30^{b}	623 ± 22^{b}	23.16
% strain	22.8 ± 0.9^{a}	25.6 ± 1.5^{a}	25.6 ± 1.5^{a}	25.4 ± 1.9^{a}	0.79
Modulus of elasticity ($kg\text{-force} \cdot mm^{-2}$)	14.8 ± 0.5^{a}	11.8 ± 0.5^{b}	11.7 ± 0.5^{b}	10.6 ± 0.6^{c}	17.28
Toughness ($g\text{-mm} \cdot mm^{-2}$)	235 ± 15^{a}	210 ± 18^{a}	207 ± 19^{a}	191 ± 20^{a}	2.21
Biochemical composition					
Collagen ($mg \cdot g^{-1}$ bone)	277 ± 2^{a}	275 ± 2^{a}	286 ± 3^{b}	281 ± 3^{ab}	2.81
Proline ($mg \cdot g^{-1}$ collagen)	167.8 ± 4.8^{a}	145.6 ± 5.4^{b}	139.1 ± 2.8^{b}	135.6 ± 5.2^{b}	9.94
Hydroxyproline ($mg \cdot g^{-1}$ collagen)	97.3 ± 1.7^{a}	94.1 ± 2.6^{a}	92.8 ± 1.9^{a}	93.6 ± 1.7^{a}	1.09
Proline:hydroxyproline ratio	1.73 ± 0.05^{a}	1.54 ± 0.07^{b}	1.50 ± 0.02^{b}	1.45 ± 0.05^{b}	6.25
Calcium ($mg \cdot g^{-1}$ bone)	301.6 ± 2.5^{ab}	294.7 ± 3.6^{b}	306.0 ± 3.1^{a}	294.3 ± 3.2^{b}	3.08
Phosphorus ($mg \cdot g^{-1}$ bone)	105.0 ± 1.4^{a}	103.2 ± 1.3^{a}	108.4 ± 1.7^{a}	108.3 ± 1.9^{a}	2.57
Calcium:phosphorus ratio	2.88 ± 0.04^{a}	2.86 ± 0.05^{a}	2.82 ± 0.03^{a}	2.72 ± 0.06^{a}	2.28
Ca + P:collagen ratio	1.47 ± 0.01^{a}	1.44 ± 0.02^{a}	1.45 ± 0.02^{a}	1.43 ± 0.02^{a}	0.64
Density ($mg \cdot cm^{-3}$)	475 ± 7^{a}	477 ± 24^{a}	473 ± 16^{a}	465 ± 8^{a}	0.13
Fish size					
Total length (mm)	332 ± 5^{ac}	319 ± 5^{c}	340 ± 6^{b}	310 ± 5^{ab}	6.15
Weight (g)	388 ± 14^{a}	371 ± 20^{a}	379 ± 20^{a}	336 ± 15^{a}	1.84
Sampled size	15	13	11	13	

TABLE 3. Mean (± SE) of metal concentrations ($\mu g \cdot g^{-1}$ dry weight) and fish size of white suckers from four Maine lakes. Means for a given measure that do not contain the same letter superscript are significantly different ($\alpha < 0.05$, $n = 5$). *F*-ratio degrees of freedom 3, 16.

	Lake and pH				
Variable	Green 7.0	Unnamed 5.9	Horseshoe 5.6	Lost 5.4	*F*-ratio
Aluminum	2.45 ± 0.75^a	6.09 ± 4.58^a	4.01 ± 0.75^a	3.18 ± 0.40^a	0.44
Cadmium	0.03 ± 0.01^a	0.03 ± 0.00^a	0.07 ± 0.00^b	0.12 ± 0.02^c	25.70
Mercury	0.14 ± 0.01^a	0.13 ± 0.00^a	0.36 ± 0.05^b	0.38 ± 0.01^b	30.32
Lead	0.66 ± 0.08^a	0.58 ± 0.04^a	2.32 ± 0.18^b	1.55 ± 0.09^c	55.60
Zinc	85.9 ± 3.7^a	58.6 ± 1.3^b	92.0 ± 2.6^a	69.6 ± 0.7^d	40.73
Total length (mm)	334 ± 5^a	308 ± 5^b	332 ± 13^a	306 ± 4^b	3.77
Weight (g)	403 ± 16^a	349 ± 23^a	361 ± 43^a	333 ± 20^a	1.199

TABLE 4. Stepwise multiple regression analysis of metal concentrations in white suckers as a function of chemical and physical variables of lake water. The best one- and two-variable models are presented for each trace metal.

Metal	R^2	*P*	Regression equation
Cadmium	0.48	0.0003	Cd = 0.27 − 0.11 Ca + Mg.
	0.65	0.0001	Cd = 0.17 − 0.07 Ca + Mg + 0.0003 Al.
Mercury	0.44	0.0007	Hg = 0.41 − 0.13 log (alk).
	0.61	0.0001	Hg = 0.53 − 0.16 log (alk) − 0.002 color.
Lead	0.58	0.0001	Pb = 2.54 − 1.0 log (alk).
	0.72	0.0001	Pb = 3.21 − 1.17 log (alk) − 0.01 color.
Zinc	0.42	0.005	Zn = 91.1 − 0.44 color.
	0.53	0.005	Zn = 122.2 + 66.4 log (Mg) − 0.40 color.

determined by automated cold vapor AAS, and zinc by flame AAS. Detection limits (micrograms per gram dry weight) were as follows: Al, 0.2; Cd, 0.005; Hg, 0.01; Pb, 0.2; and Zn, 2.0. Quality assurance measurements included analysis of U.S. National Bureau of Standards certified reference materials (liver, oyster, tuna, water), in-house reference materials (striped bass), and spiked samples.

Differences in fish metal concentrations among lakes were determined with one-way analysis of variance. For metals that differed significantly ($P \leq 0.05$) among lakes, stepwise forward multiple regression was used to test the relation of metal concentration in fish to fish size and to physical and chemical characteristics of the lakes.

Results

Green Lake differed chemically from the other three lakes (Table 1); it had a near-neutral pH (7.0), high buffering capacity (108 $\mu eq \cdot L^{-1}$), and low aluminum concentrations (28 $\mu g \cdot L^{-1}$). In contrast, the other lakes had low pH and buffering capacity, and high aluminum concentration; Lost Pond had the lowest pH (5.4) and alkalinity (5 $\mu eq \cdot L^{-1}$) and the highest aluminum concentrations (163 $\mu g \cdot L^{-1}$).

Bone Characteristics

Visual evaluation of radiographs revealed no deformed vertebrae in any fish. Bone characteristics of fish differed significantly among lakes; bones in fish from the low-pH lakes (Unnamed Pond, Horseshoe Pond, and Lost Pond) were weaker and more flexible than those in fish from the high-pH lake (Table 2). The five individual mechanical properties given in Table 2 for each location were combined into typical stress-strain curves for each location so that differences in bone mechanical properties could be readily visualized (Fig. 1). White suckers from the high-pH lake had significantly higher bone strength (rupture and elastic limit) and stiffness (modulus of elasticity) than fish from low-pH lakes. Bone strength (rupture and elastic limit) and stiffness (strain and modulus of elasticity) were highly correlated with lake water chemistry values (pH, alkalinity, calcium); the correlation coefficients (*r*) were ≥ 0.95. Waterborne concentrations of aluminum were not significantly correlated to mechanical properties of bone. The inferior mechanical properties of bone in fish from the three low-pH lakes presumably resulted from the significantly lower proline concentrations in collagen (Table 2). Proline concentrations in collagen had a correlation coefficient of 0.56 for the relation with elastic limit and modulus of elasticity, and correlation coefficients of −0.75 and −0.61 for the relation with whole-body concentrations of lead and mercury, respectively. Proline concentrations were also highly correlated with three lake water chemistry variables; pH, alkalinity, and calcium ($r \geq 0.97$).

Metal Concentrations

The mean and (in parentheses) range of percent recovery of metals from standard reference materials were as follows: Al, 100% (all 100%); Cd, 100% (100–103%); Hg, 102% (100–110%); Pb, 93% (77–100%); and Zn, 100% (99–100%). In white suckers, concentrations of all metals except aluminum differed significantly (Table 3). The pattern of metal concentrations in fish among lakes was not consistent, and the relation of metal concentrations in fish to lake chemistry were not immediately apparent. Concentrations of cadmium, mercury, lead, and zinc in fish were significantly correlated with chemical variables, but not with fish length or weight (Table 4). The most important lake chemical variables were generally the logarithm of alkalinity and the sum of calcium and magnesium.

Color was the variable most strongly correlated with concentrations of zinc in fish.

Discussion

Bone Characteristics

Skeletal deformities were not observed in the present study, although such deformities have been reported in white suckers from acidic lakes in Ontario (Beamish et al. 1975; Fraser and Harvey 1982), and can be caused by laboratory exposure to low pH (Beamish 1972; Trojnar 1977). A possible causal mechanism is the demineralization of the skeleton to maintain calcium balance for osmoregulatory and reproductive needs. Fraser and Harvey (1982) reported a 2–16% decrease in calcium concentrations in vertebrae of white suckers from three acidified Ontario lakes with pH $\leq$ 5.36 compared with concentrations in fish from lakes with higher pH. In embryos of rainbow trout (*Salmo gairdneri*) exposed to pH 4.8, ossification of the skeleton was reduced compared with that in fish from higher pH treatments, presumably due to inhibition of calcium uptake and calcium hydroxyapatite formation (Nelson 1982). Bendell-Young and Harvey (1986) reported that calcium concentrations, and concomitantly the calcium to phosphorus ratio in bone, were significantly reduced (by 8.4 and 7.3%, respectively) in white suckers from a lake with pH 4.8, but were not altered in fish from lakes with pH 5.1 – 6.4. They attributed the adverse effect on calcium concentrations in bone to possible inhibition of mineralization or replacement of calcium in the hydroxyapatite matrix of bone by elevated bone concentrations of barium, manganese, and zinc.

Altered calcium metabolism in aquatic animals exposed to low pH has been reported for crayfish (*Orconectes virilis*) (Malley 1980), brown trout (*Salmo trutta*) (Hultberg 1977), fathead minnow (*Pimephales promelas*) (Mount 1973), and white suckers (Wiener et al. 1985). Deformities of cartilaginous elements in Eurasian perch (*Perca fluviatilis*) exposed to low pH have also been reported (Runn et al. 1977). However, Lockhart and Lutz (1977) found no significant differences in calcium, magnesium, phosphorus, sodium, or potassium concentrations in rib bone of white suckers from the same lake in which deformities had been observed (Beamish et al. 1975; Fraser and Harvey 1982). Moreover, Fraser and Harvey (1982) reported that ribs and opercula were not decalcified, but calcium concentrations were reduced in centra of vertebrae; they suggested that calcium dynamics in ribs may differ from that in the vertebral column. In our study, calcium in vertebrae was not significantly lower in fish from the three low-pH lakes than in fish from the neutral lake, but there was a trend toward a reduced calcium to phosphorus ratio in fish from the low-pH lakes. In findings similar to ours, Muramoto (1981) reported that the calcium to phosphorus ratio was decreased in common carp (*Cyprinus carpio*) exposed to cadmium, thus suggesting that the increased cadmium concentrations in fish from the two lowest pH lakes in our study may have influenced mineralization processes and the structural integrity of bone.

The only significant biochemical alteration of bone observed in this study was the 13 – 19% reduction in collagen proline concentrations and a trend toward reduced hydroxyproline concentrations in collagen of vertebrae in fish from the three low-pH lakes. Altered proline and hydroxyproline concentrations in whole-fish homogenates have been observed in the fry of brook trout (*Salvelinus fontinalis*) exposed to low pH for 30 d (F. J. Dwyer, pers. comm., National Fisheries Contaminant Research Center, Route 1, Columbia, MO, 65201, USA). In Dwyer's study, proline and hydroxyproline concentrations were increased in fish exposed to low pH relative to control fish exposed to neutral pH. Although Dwyer's results differed from ours, the changes in proline and hydroxyproline concentrations observed in the two studies may indicate a biphasic response. For example, Hamilton et al. (1981b) reported that hydroxyproline concentrations in collagen were elevated in short-term exposures of channel catfish (*Ictalurus punctatus*) to toxaphene, but were decreased in longer exposures. Altered amino acid metabolism may be partly responsible for the impaired mechanical properties of bone in fish exposed to low pH.

Metal Concentrations

The significantly greater cadmium, lead, and mercury concentrations in whole-body of fish from Horseshoe and Lost ponds may have contributed to the altered bone development observed in fish from these ponds. Although we did not measure metal concentrations in bone, the elevated whole-body concentrations of cadmium, lead, and mercury suggested that their increased presence could have altered metabolic pathways associated either directly or indirectly with bone development and adversely affected the mechanical properties of bone. Moreover, cadmium and lead have been reported to concentrate in bones of fish to greater concentrations than in whole-body (Ney and Van Hassel 1983).

The accumulation of cadmium in small amounts in bone tissue of exposed mammals, greatly impairing bone development, has been reported by Itokawa et al. (1973), Bawden and Hammarstrom (1975), Yoshiki et al. (1975), and Furuta (1978). In these studies, exposure to cadmium caused morphological changes in bone such as osteoporosis, which was concomitant with reduced numbers or size of bone trabeculae. This change in the microstructure of bone suggests reduced structural integrity. Moreover, cadmium has been shown to substitute for calcium in the precusors of the crystalline inorganic component of bone mineral, calcium hydroxyapatite (Chickerur et al. 1986). Cadmium also reduces alkaline phosphatase, an important enzyme in bone calcification, and inhibits crosslinking of collagen in mammals (Bonner et al. 1980; Iguchi and Sano 1982) both of which suggest reduced structural integrity of bone. These effects on bone metabolism probably result directly from preferential accumulation of cadmium in osteoblasts (Bawden and Hammarstrom 1975), where collagen and several important enzymes are synthesized. In fish, long-term exposure to cadmium has resulted in reduced calcium and phosphorus but not magnesium concentrations in vertebra, reduced bone ash weight, and increased accumulation of cadmium in bone (Muramoto 1981). These changes in bone characteristics were significantly altered in exposed fish that were normally shaped but the changes were most predominant in deformed fish. In contrast to the findings of Muramoto (1981), Bendell-Young and Harvey (1986), who determined the concentrations of 13 metals in bone of white suckers in Ontario lakes with pH ranging from 4.8 to 6.4, reported that cadmium was not detected in fish but that the most acidic lake had fish with the highest bone concentrations of barium, manganese, and zinc. In their study, bone concentrations of aluminum, copper, iron, and nickel did not differ among lakes, and concentrations of beryl-

lium, cobalt, chromium, molybdenum, and vanadium were below their detection limits.

Lead preferentially accumulates in bone of fish (Hodson et al. 1978a; Ney and Van Hassel 1983). Moreover, the uptake of waterborne lead and its deposition in bone tissue of fish are reduced in the presence of high-calcium water or food (Varanasi and Gmur 1978). The slightly lower concentrations of calcium in waters of the three low-pH lakes examined in our study may partly explain the elevated tissue concentrations of lead. A more important factor in the higher lead concentrations in tissue was probably the low pH of Unnamed, Horseshoe, and Lost ponds relative to that of Green Lake. Low pH has been shown to facilitate the bioaccumulation of lead in fish (Merlini and Pozzi 1977), and may be a result of increased permeability of fish to lead through interaction with acid–base and ion regulation (Hodson et al. 1978b). Exposure of fish to lead can cause backbone deformities, but the deformities do not seem to be directly caused by interference in biochemical metabolism of bone; rather, they seem to be caused indirectly by neuromuscular interactions (Holcombe et al. 1976; Hodson et al. 1980). The deposition of lead in bone may have also altered its structural integrity.

Exposure of fish to mercury may adversely affect bone development, causing spinal deformities and other abnormalities (Heisinger and Green 1975; Weis and Weis 1977; Sharp and Neff 1980; Snarski and Olson 1982). However, these effects are generally observed at exposure concentrations exceeding those causing other sublethal effects such as inhibited reproduction and reduced growth. Mercury has a high affinity for sulfur-containing amino acids, proteins, and enzymes and thus expresses its toxicological effect as a general systemic inhibition of the functional role of the affected ligand (Vallee and Ulmer 1972).

Elevated whole-body concentrations of cadmium, lead, and mercury or low pH of lake water, either individually or combined, could have caused the inferior mechanical properties of bone or the altered proline concentration in collagen of white suckers from the three low-pH lakes. However, other factors that were not measured in our study, such as diet, may have influenced bone development and contributed to the measured differences in bone characteristics. Nevertheless, the changes in bone characteristics of fish we examined, especially changes in mechanical properties, suggested that white suckers inhabiting acidified lakes are less fit to compete for food or habitat or avoid predators, because the spinal column is the major anchor for the musculature used in swimming. Vertebrae with reduced strength and elasticity would be less likely to withstand stress and strain forces imposed on them by the musculature during swimming activity, especially during burst swimming which is associated with predator–prey interactions or territory defense. Nelson (1982) also came to this conclusion when he stated "Organisms (i.e. fish) in acid water could be relatively frail (i.e. reduced ossification of skeletal parts), rendering them more susceptible to environmental damage and predation." Measurement of bone composition, density, and (especially) mechanical properties in fish may have potential as a monitoring tool for acidic environments.

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Evaluation of Variation in Permeability Measurements when Using the MARK VI Standpipe

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Using the MARK VI standpipe in a substrate-filled flume under constant flow conditions, we found significant differences in permeability readings made by different people at four of five sites. Coefficients of variation at each site ranged from 27 to 79%. Readings usually varied greatly for each person at each site. To detect a 10% change in the mean permeability at single sites ($\alpha = 0.05$) would require from 34 to 90 samples; for a 30% change, the range was 4 – 10. Repeated sampling at a site did not produce directional changes in permeability estimates, nor did the performance of individuals change during the test. We propose a sampling strategy based on repeated readings taken by one person.

Avec une colonne montante MARK VI dans un canal rempli d'un substrat et où l'écoulement est régulier, on a observé des différences significatives dans les lectures de perméabilité faites par différentes personnes à quatre stations sur cinq. Les coefficients de variation par station s'étalaient entre 27 et 79 %. Habituellement, les lectures variaient considérablement selon chaque personne et selon chaque station. Pour détecter une variation de 10 % de la perméabilité moyenne à des stations uniques ($\alpha = 0{,}05$), il fallait 34 à 90 échantillons. Pour une variation de 30 %, il en fallait de 4 – 10. Des échantillonnages répétés à des stations données n'ont pas produit d'indications de changements directionnels dans les évaluations de la perméabilité, et la performance des personnes qui ont fait les mesures n'a pas changé durant le test. Nous proposons une stratégie d'échantillonnage fondée sur des lectures répétées par une seule personne.

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The impact of fine sediment on salmonids has been studied for more than 60 yr (Harrison 1923). In a relation that is not precisely understood, increases in fine sediment lead to decreases in the embryonic intragravel survival of numerous species (Cordone and Kelly 1961; Iwamoto et al. 1978). Among the suggested influences of fine sediment on spawning substrates is a reduction in permeability (Cooper 1965).

Permeability and hydraulic head directly determine intragravel water velocity, as demonstrated by Darcy's Law (Pollard 1955). Estimates of permeability have focused on two methods, laboratory estimates based on permeameters (McNeil and Ahnell 1964) and field estimates based on the use of standpipes (Gangmark and Bakkala 1958). Because the laboratory estimates require that substrate be removed from a streambed, the true permeability cannot be measured because the substrate loses its instream arrangement and compaction (Pollard 1955). Consequently instream measurement of permeability is preferred.

Wickett (1954), Pollard (1955), and Terhune (1958) developed the standpipe method to measure permeability of the spawning substrates of salmonids. By applying suction in the standpipe at a point 2.5 cm below the surface of the water inside the pipe, the operator draws water through the perforated tip buried in the substrate. The volume of water collected is an index of the permeability of the substrate. Terhune (1958) demonstrated remarkable precision with this technique; coefficients of variation ($c(v)$ = standard deviation/mean × 100) ranged from 1 to 5%. In contrast, Pollard (1955) found greater scatter about the predicted mean permeabilities, and his test substrates seemed much more representative of salmonid redds than those of Terhune.

After evaluating the available techniques for directly or indirectly evaluating intragravel flow in salmonid redds, Chapman and McLeod (1987) suggested that the standpipe measurement of permeability was the most desirable method. Previous studies of salmonid spawning habitats have relied on this method of permeability estimation (Wickett 1958; Turnpenny and Williams 1980). Several investigators (e.g. Coble 1961; Hansen 1975) failed to demonstrate a relation between permeability and survival of salmonid embryos to emergence, despite the theoretical support for this relation (Shumway et al. 1964; Vaux 1968). Few researchers have reported means and variances for their measurements, apparently assuming that single readings were adequate descriptors of permeability (Koski 1966; Reiser and Wesche 1977). Our field observations of this technique

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TABLE 1. Means of permeability readings ($mL \cdot s^{-1}$) for each combination of person and sampling site. All cell means are based on three observations per cell except cells I–3 (one observation) and V-1 (two observations). Sample standard deviations are given in parentheses.

	Site				
Person	1	2	3	4	5
I	2 (1)	23 (5)	12 (0)	27 (16)	38 (12)
II	4 (2)	15 (7)	57 (5)	45 (4)	38 (3)
III	5 (5)	21 (7)	42 (3)	39 (2)	23 (10)
IV	2 (2)	12 (9)	42 (6)	30 (15)	33 (6)
V	4 (0)	25 (2)	40 (4)	39 (2)	32 (5)
Means	3 (3)	19 (7)	43 (12)	36 (11)	33 (9)

made us less certain of its precision and led to the present laboratory study.

We wished to assess the variability in permeability estimates made by different people, at different sites, and through time under laboratory conditions. By replicating measurements by individuals at given sites, we hoped to estimate the precision of this technique.

Materials and Methods

We conducted the experiment from 0800 to 1200 on 18 May 1987. We used a 21.3-m flume, containing a substrate typical of that found in the Big Laramie River of southeastern Wyoming, in the Hydraulics Laboratory of the University of Wyoming Department of Civil Engineering. The materials and technique used for determining permeability closely followed those used by Terhune (1958). We drove a standpipe 25 cm into the substrate once at each of five randomly selected sites that had different substrate compositions and hydraulic characteristics (i.e. pools or riffles). Flow through the flume was $0.07\ m^3 \cdot s^{-1}$. Water temperature was held constant (at 18°C), since changes in water temperature alter viscosity and hence permeability (Terhune 1958).

Five people, with varying physical abilities and differing amounts of experience in using this technique, collected the samples. To collect a sample, a person pumped water for 5 or 10 s through a copper tube (inside the standpipe) into a graduated cylinder using a modified bicycle pump (Terhune 1958). The workers usually took three samples at each site; the sequence of sampling for each person and each site was randomized. Because one person took only one reading at site 3 and two readings at site 1, the total number of readings was 72. All analyses were performed on a standardized sample of discharge ($mL \cdot s^{-1}$) into the graduated cylinder, less the 25 mL introduced by the sampling technique. We used two-way ANOVA, performed by the GANOVA-4 program (Courtesy of D. G. Bonett, Department of Statistics, University of Wyoming, Laramie, USA), to assess differences between people and sites; pairwise comparisons were made between all individual sampler combinations within sites and across all sites. Tests for runs up and down and binomial probabilities (Mosteller and Rourke 1973) were used to determine if permeability changed at each site through time. Finally, we calculated estimates of the sample sizes needed to detect possible changes of 10, 20, or 30% in the permeability means at each site (Sokal and Rohlf 1981). An α of 0.05 was accepted as indicating significance.

TABLE 2. Pairwise comparisons of samples taken by different individuals at each site based on *F*-tests (NS indicates no significant difference; * indicates significance at an $\alpha = 0.05$; ** indicates significance at an $\alpha = 0.01$).

		Person			
Site	Person	I	II	III	IV
1	II	NS			
	III	NS	NS		
	IV	NS	NS	NS	
	V	NS	NS	NS	NS
2	II	NS			
	III	NS	NS		
	IV	NS	NS	NS	
	V	NS	NS	NS	*
3	II	**			
	III	**	*		
	IV	**	*	NS	
	V	**	**	NS	NS
4	II	**			
	III	*	NS		
	IV	NS	**	NS	
	V	*	NS	NS	NS
5	II	NS			
	III	*	*		
	IV	NS	NS	NS	
	V	NS	NS	NS	NS

TABLE 3. Number of permeability samples necessary to detect potential changes of 10, 20, or 30% in the mean permeability ($\alpha = 0.05$) at the five sites.

	Sites					
Potential change (%)	1	2	3	4	5	Mean
10	90	68	38	44	34	55
20	23	17	9	11	9	14
30	10	8	4	5	4	6

Results

Mean permeability readings ranged from 3 to 43 $mL \cdot s^{-1}$ over the five sites; coefficients of variation at each site ranged from 27 to 79%. Means between individuals varied greatly within and between sites (Table 1). We were unable to detect overall differences among people by applying two-way analysis of variance, but we did identify significant differences among sites. A significant interaction between people and sites suggested that people performed differently at different sites. Nonetheless, when we transformed the measured values to ranks, we found the majority of people gave the same rank to a given site.

Pairwise comparisons of samples withdrawn by different people across all sites yielded only one significant difference (between samples collected by persons I and II), but pairwise comparisons of samples taken by different people at each site revealed several significant differences (Table 2). Only for site 1 did we find no significant differences between the samples removed by all possible pairs of individuals, which may be attributable to the variance associated with samples at this site rather than to a lack of differences between people. At site 3, seven of the 10 pairwise comparisons were significant, but a low single reading by sampler 1 influenced this result. None-

theless, the samples withdrawn by each individual differed significantly from those taken by at least one other person at one or more sites.

Permeability did not exhibit a directional shift at any site during the experiment (test for runs, $P > 0.05$). The probabilities that readings by specific samplers at each site had successively increased (sample 1 < sample 2 < sample 3) or decreased (sample 1 > sample 2 > sample 3) were not significant. The number of samples needed to detect a 10% change in the mean permeability of a site varied from 34 to 90, to detect a 20% change, from 9 to 23, and to detect a 30% change, from 4 to 10 (Table 3).

Discussion

We demonstrated the need to take replicate samples for permeability estimates. Yet single samples taken at various intervals have been used to evaluate temporal variation in permeability (e.g. Reiser and White 1981). Typically, researchers attributed this variation to fluctuation in permeability, rather than to imprecision of the technique and the individual sampler (Moring 1982). Furthermore, sampling by different people at a single site should be cautiously interpreted, and we question the comparison of permeabilities between different streams when readings are collected by different people whose sampling biases are unknown (e.g. Moring 1982).

Terhune (1958) stated that the probable error in predicting mean permeability when using his calibration curve was 1.1%, and that a liberal allowance for error would be 10%. Conversion of our samples to permeability estimates by using the figure published by Terhune (1958) exacerbated the variability, since the calibration curve is based on a log–log plot and one performs the conversion graphically. Thus we believe that the standpipe technique is best adapted to assessing relative differences or changes, rather than providing precise estimates of permeability. Replicated readings by one person taken at various sites or times will probably reflect true changes in permeability.

Pollard (1955) suggested that permeability readings did not change over the course of sampling. Our results confirm this conclusion and suggest that individual samplers behaved consistently during the test, despite the strenuous sampling effort. From these perspectives, the standpipe method has potential for detecting temporal or spatial changes in permeability.

Proposed Sampling Strategy

We demonstrated limitations of the standpipe technique for measuring permeability, but we also identified advantages of this method and believe it can be successfully used to assess permeability. We offer the following sampling strategy:

(1) Drive a single standpipe into the specific location of interest, e.g. the egg pocket of a salmonid redd. If spatial differences are of interest, the standpipe can be removed after sampling. If temporal changes are of concern, then the standpipe should not be removed or disturbed because doing so could alter future readings.

(2) Select the percentage change in the mean permeability that one wishes to be able to detect. Our results suggest that about 15 samples should enable one to recognize a 20% change in mean permeability at an α of 0.05, but variability among sites may require a different number of samples to detect a similar change.

(3) One person should collect all the readings, following the technique developed by Terhune (1958). Intervals between sampling may vary, but all samples should be collected at similar water temperatures and flows.

(4) Results should be interpreted as an index of permeability, rather than as an accurate estimator.

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Gut Sediments in a Burrowing Mayfly (Ephemeroptera, *Hexagenia limbata*): Their Contribution to Animal Trace Element Burdens, Their Removal, and the Efficacy of a Correction for Their Presence

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Hare, L., P. G. C. Campbell, A. Tessier and N. Belzile. 1989. Gut sediments in a burrowing mayfly (Ephemeroptera, *Hexagenia limbata*): their contribution to animal trace element burdens, their removal, and the efficacy of a correction for their presence. Can. J. Fish. Aquat. Sci. 46: 451–456.

Nymphs of the burrowing mayfly *Hexagenia limbata* were collected in the field, returned to the laboratory, and dissected to remove the gut contents. Concentrations of four trace elements (As, Cd, Cu, Zn) were determined both for the gut contents and for the body. Trace elements in gut contents represented up to 22% of whole animal trace element burdens. Studies of depuration of *H. limbata* nymphs held in water for up to 48 h indicate that individuals vary substantially in the rate at which they egest gut contents and that 48 h is not sufficient to ensure a complete emptying of the gut. A model developed to compensate for the presence of gut contents in the determination of body trace element burdens was applied to *H. limbata*. Comparisons of model estimates with actual body burdens (without gut contents) show that the model accurately predicts As and Cu concentrations, but overestimates concentrations of Cd and Zn by as much as 20%. We suggest that the biases in the model result from assuming that: (a) trace element concentrations in gut sediments are equivalent to those in sediments sampled from the animal's surroundings, and (b) there is no weight loss of gut contents during sample digestion (a two-thirds weight loss of gut contents was in fact observed). These biases may be minimized by, respectively: (a) measuring trace element burdens of gut contents and (b) compensating for weight loss of gut contents during digestion.

Des nymphes de l'éphémère fouisseur *Hexagenia limbata* ont été recueillies, rapportées au laboratoire et disséquées de façon à retirer le contenu intestinal. Quatre éléments traces (As, Cd, Cu, Zn) ont été dosés dans le contenu du tractus intestinal et dans le corps de l'organisme. Les quantités retrouvées dans le contenu du tractus représentaient jusqu'à 22% de celles présentes dans l'organisme. Les études de dépuration chez les nymphes de *H. limbata* maintenues dans l'eau pendant 48 h montrent que le taux d'excrétion du contenu du tractus intestinal varie substantiellement d'un sujet à l'autre et que 48 h ne suffisent pas à assurer l'évacuation complète du tractus. Un modèle mis au point pour compenser pour la présence du contenu du tractus intestinal lors de l'évaluation des quantités d'éléments traces dans l'organisme a été appliqué à *H. limbata*. La comparaison des estimations fournies par le modèle avec les quantités mesurées dans le corps (sans le contenu du tractus intestinal) montre que le modèle prédit correctement la concentration en As et en Cu mais conduit à une surestimation de la concentration en Cd et Zn, jusqu'à 20% parfois. Nous supposons que les erreurs systématiques du modèle sont le résultat du fait que les hypothèses suivantes sont admises: (a) les concentrations en éléments traces dans les contenus du tractus intestinal sont équivalentes à celles observées dans les sédiments prélevés dans le milieu où vit l'animal, et (b) le contenu du tractus intestinal ne perd pas de poids lors de la digestion de l'échantillon (une perte de poids correspondant aux deux tiers a été observée). Ces erreurs systématiques peuvent être minimisées de la façon suivante: (a) en mesurant les quantités d'éléments traces dans le contenu du tractus intestinal et (b) en compensant pour la perte de poids du contenu du tractus durant la digestion.

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Compared with the ephemeral nature of chemical conditions in lake water, the relatively long life span and reasonably sessile nature of benthic organisms make them attractive as continuous biological monitors of pollutants. Benthic animals are known to accumulate pollutants and can effect their transfer from sediments. A common source of error in determining pollutant levels in benthic organisms is the presence in the digestive tract of pollutant-containing material which may or may not be ultimately incorporated into the organism. Thus a variety of techniques have been developed for compensating for gut contents when body burdens of pollutants are measured. Gut contents may be removed directly by dissection or by placing the live animal in water or in uncontaminated sediments for a length of time sufficient to eliminate its gut contents (depuration). Alternatively, the contaminant level in the whole undepurated animal may be measured and an estimate of the proportion contributed by the gut contents subtracted. Chapman et al. (1980) used such a correction technique for estimating the concentrations of trace metals in Oligochaeta (Annelida). The weight of residue remaining after chemical digestion of the organisms was multiplied by the concentration of the trace metal in the sediments from which the oligochaetes

had been collected. This quantity of trace element was then subtracted from the whole animal trace element burden.

Each of these methods has drawbacks. Dissection may not be possible on very small organisms, or where very large numbers of specimens are required. In addition, pollutant losses and cross-contamination of tissues can occur. Depuration in water may be very slow or incomplete for animals requiring incoming food to push the gut contents through. Depuration in sediments, though probably more rapid than in water, may be difficult to verify unless contaminated and uncontaminated sediments can be visually distinguished. An additional difficulty with depuration is the extreme change in the contaminant concentration gradient between the organism and its surroundings, with the concomitant potential for contaminant loss from the organisms. Laboratory results suggest that losses of some metals by animals held in water without nourishment may be rapid, e.g. larvae of *Chironomus* can lose 50% of their body burden of Cd over 3 d (Yamamura et al. 1983). Losses could be particularly acute in small animals, i.e. those with high surface to volume ratios.

Despite these limitations, very few investigators have attempted to establish the errors associated with either the presence of gut contents or the various methods of their removal. In this paper, we compare the body trace element burdens of *Hexagenia limbata* (Serville) (Ephemeridae, Ephemeroptera, Insecta) determined by dissection with those determined by the compensation model of Chapman et al. (1980). We also describe the pattern of material loss from the gut during depuration in water.

Materials and Methods

Collection of Nymphs

Nymphs of the burrowing mayfly *Hexagenia limbata* were used in the experiments. The species was identified by the examination of nymphs and laboratory-reared imagoes using the descriptions of McCafferty (1975) and Spieth (1941), respectively. Animals were collected on 21 January 1987 from Lake Joannès (surface area 4.5 km^2) situated 25 km to the east of the mining city of Rouyn-Noranda in northwestern Quebec (48°11′N, 78°41′W). The major source of trace element contamination to the lake is by atmospheric fallout from smelter emissions. Nymphs were collected at a mid-lake site, at a depth of 15 m, with a 15 × 15 cm Ekman grab. The population density of nymphs at this site on the sampling date was 310 ± 20 nymphs·m^{-2} ($\bar{X} \pm$ S.E., $n = 4$ grabs). Animals were transported to the laboratory, live, in closed plastic sacs containing lake sediments and water maintained near field temperature (2°C). In the laboratory, nymphs were kept in sediments from the collection site, in the dark, at a temperature of 4°C, for a period of 7 wk prior to dissection. During this time various methods of depuration, dissection, drying, and chemical digestion were tested.

Depuration, Dissection, and Digestion of Nymphs

In order to estimate the contribution of gut contents to animal trace element burdens, 10 nymphs covering a wide range of sizes (0.7–2.0 mm in length, excluding cerci) were sieved individually from sediment and were dissected immediately in small amounts (2 mL) of deionized water under a microscope at magnifications of 120 and 250×. An animal was immobilized by cutting its nerve cord by means of a ventral incision between the head and thorax (which also served to sever the intestine), then the seven pairs of plumose gills were plucked from the abdomen. A mid-ventral incision was made along abdominal segments 7–9 which permitted the extraction of the digestive tract from the posterior end. Once extracted, the anterior end of the gut was torn open, so as to expose the tube of compacted material within. One pair of forceps was used to hold the gut while the contents were withdrawn with another pair. If the cylinder of compacted gut contents broke during removal, an incision was made at the site of the break in order to withdraw

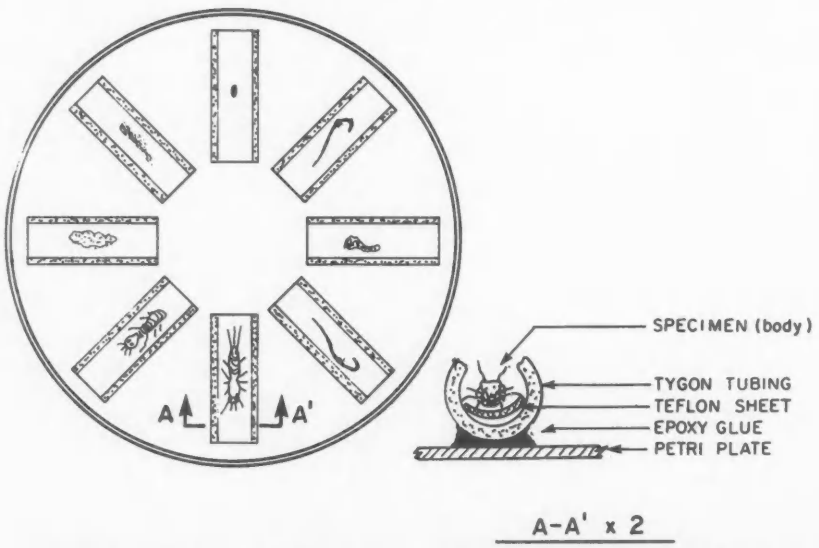

FIG. 1. Apparatus for drying small animal parts; consisting of a plastic petri dish onto which are glued half cylinders of Tygon tubing, into which pieces of Teflon sheet are placed to hold animal parts.

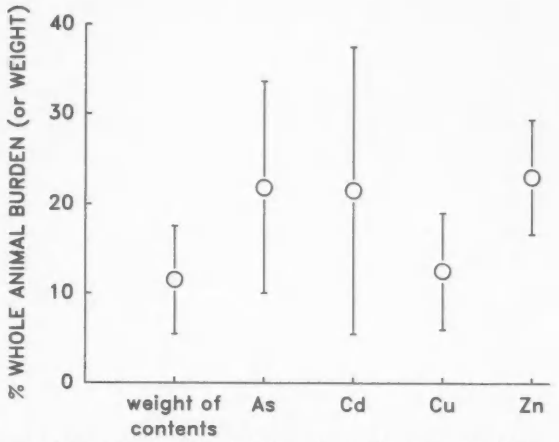

FIG. 2. Contribution (%) of weight and trace element levels of the gut contents of *Hexagenia limbata* to those of the whole animal; $\bar{X} \pm SD$, $n = 10$.

the remainder. A separate incision was made in the region of the gut posterior to the junction of the Malpighian tubules for removal of the contents therein. Animals whose guts broke up during dissection were discarded. As each part of the animal was removed (gills, gut, gut contents, remainder of body) it was placed for drying on a preweighed piece of acid-washed Teflon of appropriate size (thickness 0.013 mm; Warehoused Plastic Sales, Toronto, Ontario, cat. #615) which was held in a longitudinally cut piece of Tygon tubing cemented onto a plastic petri dish (all acid-washed), as illustrated in Fig. 1 (modified from method of N. Yan, Ontario Ministry of the Environment, Dorset, Ontario, pers. comm.). When necessary, a Pasteur pipette was used to transfer scattered material from the gut onto the Teflon piece. This apparatus facilitates the handling and drying of many small samples simultaneously; the pieces of Tygon tubing act to hold samples in place, the placement of samples on Teflon minimizes the possibility of their contamination, and the light weight of the Teflon pieces permits the accurate measurement of sample weights.

The covered petri dish was held in a freezer at −40°C (at least 30 min) and then in a lyophiliser (at least 12 h) in order to dry its contents. Each piece of Teflon holding a body part was weighed with a Mettler ME30 electronic micro-balance (balance pan covered with Teflon) and then placed into a thick-walled, screw-cap, acid-washed Teflon vial (Nalgene; 7.5, 15, or 30 mL capacity, according to specimen weight). Concentrated nitric acid (Aristar purity) was added to each vial (100 μL for each 1 mg tissue dry weight) which was then closed and heated in a water bath at 70°C for 48 h. Small samples (5–15 mg) of a certified standard (lobster hepatopancreas — Tort 1, National Research Council of Canada) were digested at the same time as the *Hexagenia* samples for use in verifying the accuracy of the entire procedure. Measured values of this standard were within the range of certified values for each of the elements studied. After digestion and analysis (see below), gut contents were filtered through acid-washed, preweighed, 0.4-μm Nuclepore filters. These were dried at 60°C to a constant weight and the weight of the filtered material calculated by subtraction. In addition, surface oxic sediments for trace element analysis (four samples of 2–5 mg dry weight) were collected with a pipette from the aquaria containing *H. limbata* and were dried and submitted to the digestion procedure.

TABLE 1. Mean concentrations ($\pm$ SD, $\mu g \cdot g^{-1}$) of various trace elements in the gut contents of nymphs of *Hexagenia limbata* ($n = 10$) and in the surficial lake sediments in which the nymphs live ($n = 4$) and the ratio of the latter to the former.

	As	Cu	Cd	Zn
[TE] in gut contents	39 ± 12	63 ± 9	18 ± 13	530 ± 170
[TE] in sediments	79 ± 5	91 ± 2	4 ± 0.1	290 ± 13
[TE] in sediments / [TE] in gut contents	2.01	1.44	0.23	0.55

For the measurement of changes with time in the weight of gut contents, nymphs were taken from sediments (at 5°C) and placed in 5°C lake water, which was either maintained at 5°C or allowed to equilibrate to room temperature (25°C), for up to 48 h. Coprophagy was minimized by placing netting (1-mm mesh aperture) in the water-filled containers which allowed faeces to fall to the container bottom out of reach of the nymphs. Dissection, drying, and weighing of nymphs followed the above described procedure except that the body parts of each nymph were dried and weighed together, only the gut contents being weighed separately.

Analytical Procedures

All labware was soaked in 15% nitric acid for 24 h and rinsed in deionized water prior to use. Deionized water for all uses was obtained from a Milli-Q3RO/Milli-Q2 system (Millipore Corp.). Manipulations of samples were performed whenever necessary in a laminar flow hood.

Cooled digested samples were diluted with deionized water to a concentration of 16.6% nitric acid (5:1 v/v). Trace element concentrations of the various solutions were measured with either a Varian AA-1275 series atomic absorption spectrophotometer equipped with a Varian GTA-95 graphite tube atomizer (As, Cd, and Cu) or a Varian AA-575 series flame atomic absorption spectrophotometer (Zn).

Results and Discussion

Contribution of Gut Contents to Weight and Trace Element Burdens of the Whole Animal

Gut contents collected from nymphs immediately after their removal from sediments represented a mean of 12% of the weight of whole animals (including gut contents) (Fig. 2). This proportion varied substantially among individuals (range of 2.5–22.0%) and showed little relation to animal dry weight (linear regression; $r^2 = 0.08$). Gut contents of *Hexagenia limbata* represented means of 12% (Cu) – 22% (As, Cd, and Zn) of whole animal trace element burdens (Fig. 2). These proportions were significantly greater than that for gut contents by weight for both As ($P = 0.025$) and Zn ($P = <0.01$) but not for Cd ($P = 0.10$) and Cu ($P = 0.85$) (Wilcoxon's signed-ranks test). These differences among elements are related to the ratios of the concentrations of these trace elements in the gut contents (Table 1) to those in the animal (Table 2), which were about 2.0 for As and Zn and 1.0 for Cu; although the ratio for Cd was 1.5, great variability among nymphs rendered insignificant the difference between the weight and trace element burden proportions (as compared above and in Fig. 2).

TABLE 2. Mean concentrations ($\pm$SD, $n = 10$) of various trace elements in the entire body (excluding gut contents) of *Hexagenia limbata* and the percent change in these means if: (a) the sediment concentration of a trace element, $[TE]_s$, is used to estimate that in the gut contents, $[TE]_g$; (b) the weight of residue remaining after digestion of the whole animal in HNO_3 is used to estimate the original weight of the gut contents (W_g); (c) both of these factors are applied. Significance of differences between measured values (row 1) and calculated values (a, b, and c) was assessed using Wilcoxon's signed-ranks test where: **, $P < 0.01$; *, $0.01 < P < 0.05$; *ns*, $P > 0.05$.

	As	Cu	Cd	Zn
[TE] measured in body ($\mu g \cdot g^{-1}$)	22 ± 11	62 ± 13	12 ± 8	244 ± 13
(a) Percent change in mean body [TE] if gut content [TE] taken as sediment [TE]	−28%**	−13%**	+15% ns	+3% ns
(b) Percent change in mean body [TE] if weight of gut contents taken as that remaining after digestion	+10%*	+1% ns	+13% ns	+9% **
(c) Percent change in mean body [TE] if both factors (a) and (b) are applied	+1% ns	−1% ns	+20% *	+13% **

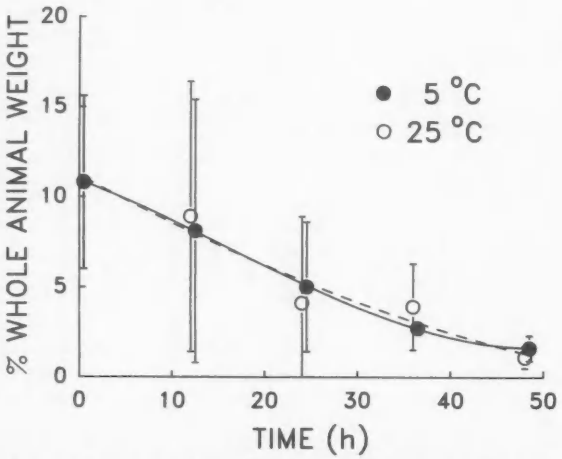

FIG. 3. Proportion of whole animal dry weight represented by gut contents for nymphs of *Hexagenia limbata* which had been removed from sediments (at 5°C) and kept in water (at either 5°C or 25°C) for various lengths of time; $\bar{X} \pm$ SD; $n = 10$ (time 0), 4 (time 24 h), or 3 (others). Second-order curvilinear regression lines are plotted for the two temperatures (5°C solid line, 25°C broken line).

Studies on other benthic animals suggest that gut contents can account for even higher proportions of animal pollutant burdens; e.g. gut contents can represent >60% of Pb and Ni body burdens of oligochaetes and ammocoetes larvae (Chapman 1985). Thus the accurate estimation of animal pollutant burdens usually necessitates the removal of gut contents, or at least a compensation for their contribution to animal burdens. An exception might be in the study of predator–prey relationships where pollutants in prey gut contents contribute to the predator's pollutant intake and thus should be included in prey analyses. Where desirable, contaminated gut contents can be eliminated by dissection, by depuration of the animals in water, or by replacement of gut contents with less contaminated food (e.g. by depuration in clean sediments). Alternatively, compensation can be made for the contribution of gut contents to body pollutant burdens. The merits and drawbacks of each of these approaches for the accurate determination of trace element body burdens are discussed below.

Dissection

Dissection of animals permits the direct and complete removal of gut contents and allows the determination of the distribution of pollutants among body parts. The major drawbacks of dissecting to remove gut contents are: it is time consuming and thus may be impractical when dealing with large numbers of animals; it can not be used for very small animals; cross-contamination among body parts can occur (Uthe and Chou 1987); and there could be a loss of body fluids (including associated pollutants) during dissection. To evaluate this latter point, losses of trace elements during dissection in water were measured by labelling nymphs of *H. limbata* (50-d exposure) in study-lake sediments containing the radio-isotopes ^{109}Cd, ^{210}Pb, and ^{65}Zn (added to sediments one year prior to use). Animals were depurated in nonradioactive sediments prior to dissection. Measures of these radio-isotopes in the water remaining after dissection represented, respectively, $2.8 \pm 3.9\%$, $0.4 \pm 0.1\%$, and $0.7 \pm 1.3\%$ of the mean totals present in the animals ($n = 7$). These results suggest that trace elements are associated in large part with body tissues and thus are not readily lost in body fluids during dissection.

Depuration

The proportion of whole animal dry weight represented by gut contents declined progressively over time during depuration in water (Fig. 3). From an initial mean of 12%, gut contents came to represent less than 2.2% of total body weight after 48 h. However, gut contents represented a highly variable proportion of total animal weight during the depuration period, as evidenced by the large standard deviations about the means, notably at 12 h (Fig. 3).

The times required for complete depuration under various conditions have not been measured for most benthic animals, but periods of 6–48 h have been commonly used, e.g. Pruell et al. 1986 (6 h); Lewis and McIntosh 1986 (24 h); Luoma and Jenne 1977 (48 h). During depuration animals should be inhibited from coprophagy by the placement of netting in the depur-

ation container (as in this study) or by frequent changes of water. As individuals of *H. limbata* can vary greatly in the rate at which they empty their guts (after 12 h of depuration, both individuals with empty and full guts were found), a depuration duration of less than 48 h could actually increase the variability among nymphs in terms of the quantity of gut contents present and thus their apparent trace element burdens. Complete depuration in water could take much longer than 48 h for species that require incoming food to push the gut contents through; e.g. we observed that *Chironomus* spp. larvae and some *H. limbata* nymphs held in water for 2 wk did not clear their guts completely. If trace element concentrations are high in the gut contents compared with those in the body, the persistence of even small quantities of material in the gut (as occurred after 48 h in this study) could bias measures of body trace elements.

Animals taken from the field are usually depurated at either field or room temperatures. Results of this study suggest that animals taken from sediments at 5°C and placed in water at either 5°C or 25°C differ little in the rate at which gut sediments are expelled (cf. similarity of regression lines, Fig. 3). This is not to suggest that in nature gut passage times of invertebrates would be unaffected by temperature. Nymphs of *H. limbata* taken from the field at various times of the year, and depurated at field temperatures in the laboratory, showed a negative relationship between temperature and gut clearance time (Zimmerman and Wissing 1978).

Depuration over a period of several days could lead to the loss of pollutants from an animal. For a given pollutant, rates of such losses could vary among individuals of the same or different species, depending on their sizes, surface area to volume ratios and metabolic rates. Thus, not only might the total pollutant concentration be affected, but the variability among individuals could be increased as well. For sediment feeders, depuration of contaminated gut contents can probably be achieved in a much shorter time by allowing animals to feed on little-contaminated natural or artificial sediments which would act to push through contaminated material. For *H. limbata*, gut clearance times of approximately 5 h in natural sediments (at 20°C) have been reported (Zimmerman and Wissing 1978). Even shorter gut passage times probably occur in nature (Hare, unpubl. data).

Difficulties of depuration in sediments, as opposed to water, can lie in; (a) obtaining sediments or artificial substrates which are consumed readily and which are sufficiently low in pollutant concentrations compared with those of the animal (in the case of lightly contaminated animals), (b) ensuring that an exchange of gut contents has taken place, and (c) measuring gut-content-free body weight (depurated animals will still contain gut contents) for use in calculating body pollutant concentrations.

Correction for the Presence of Gut Contents

An indirect means of compensating for the presence of gut contents in the determination of animal pollutant burdens is to measure pollutant burdens in whole animals (including gut contents) and to correct for the presence of gut contents. As individuals are likely to vary in the fullness of their guts (e.g. SD in Fig. 2), such a correction would be best applied on a sample-by-sample basis and not as a single mean correction factor for all samples of each taxon. The efficacy of a correction factor used by Chapman et al. (1980) for trace metals (equation 1), which corrects for gut contents on a sample-by-sample basis, has been evaluated in the present study. Direct measures of trace elements in the body and in gut contents removed by dissection have been used to assess its accuracy. It can be summarized as:

$$(1)\quad [\mathrm{TE}]_b = \frac{W_t \cdot [Te]_t - W_g \cdot [TE]_s}{W_t - W_g},$$

where [TE] = trace element concentration (micrograms per gram), W = weight (grams), and the subscripts refer to: (b) the entire body (gills + intestine + remainder, but excluding gut contents), (t) the whole animal (total including gut contents), (g) the gut contents, and (s) the sediments in which the animal lived. Direct measures of mean entire body concentrations of various trace elements in *H. limbata* are presented in Table 2 (row 1). The extent and significance of differences between these direct measures, and estimates made according to equation (1) (Table 2, rows *a–c*), will be discussed. Two sources of error that are inherent in the correction method require evaluation.

The first source of error lies in the use of the sediment concentrations of trace elements to approximate those of the gut contents. The assumption that sediment collected by an investigator corresponds in character to that eaten by an invertebrate ignores the substantial heterogeneity of bottom sediments. Trace elements are not usually distributed in lake sediments in a homogeneous manner, concentrations usually differing between sediments in the surface oxic and deeper anoxic layers (Carignan and Nriagu 1985). Furthermore, trace elements may be differentially associated with particles according to their size and/or quality (Warren 1981). Thus species that concentrate their feeding at different depths in the substrate or on different particle sizes or types, or species that show no electivity in feeding, could consume sediments having substantially different trace element concentrations. Even with such knowledge, it will be difficult for an investigator to sample the same quality of sediment as that contained in an animal's gut.

In the present study, the concentrations of trace elements in the neighbouring oxic sediments were higher than those measured in gut contents for two of the elements (As, Cu) and lower for the two others (Cd, Zn) (Table 1). Substituting the concentrations of trace elements in the sediment for those measured directly in the gut contents biases the resultant estimates either in a negative (As, Cu) or in a positive (Cd, Zn) direction (Table 2, a), as would be expected from the ratios of trace element concentrations in sediment to those in gut contents (Table 1). The positive bias for Zn is small and not statistically significant, while that for Cd, although larger, is not statistically significant due to the great variability of Cd concentrations among nymphs. Since the amount and direction of the bias vary among elements, no simple correction factor could be used to take this into account. Where possible, it would be preferable to measure directly the pollutant concentrations of gut contents, either removed from animals by dissection or collected after their depuration in pollutant-free water, rather than use an indirect measure of pollutant concentrations in neighbouring sediments.

A second source of error inherent in corrections with equation (1) lies in equating the weight of undigested gut contents with the weight of residue remaining after digestion of the whole animal. Since a proportion of the gut contents will be dissolved during the digestion, the weight of the residue will be less than that contained initially in the gut, leading to an underestimation of the gut content trace element levels and an error in those for

the body itself. In the present study, gut contents removed from the body by dissection were weighed and digested and their trace element contents measured. The subsequent filtering and reweighing of these samples gave a mean weight after digestion that was only 32 ± 7% (± SD) of initial mean gut content weight. For any one digestion procedure, the extent of such weight loss would depend in large part on the character of the sediments consumed, e.g., organic matter and carbonate contents. For example, the profundal lake sediments studied herein had a total organic carbon content of 5.7 ± 0.3% ($\bar{X}$ ± SD, n = 3) and were very low in carbonates. Since these components are the ones most likely to be solubilized by the digestion method used, the loss of two-thirds of the weight of gut contents during chemical digestion supports the idea that *H. limbata* sorts sediment so as to increase the quantity of organic matter consumed. Consequently, gut contents and surrounding sediments may differ greatly in composition, as suggested above.

Use of the weight of digested gut contents, as proposed in the correction method, increases the apparent mean body concentrations of the majority of elements studied (Table 2, b). There was little or no change in values of Cu, as the mean concentration of this element in gut contents represented a lower proportion of that for the whole animal than was the case for the other trace elements (Fig. 2). Measures of Cd varied greatly among nymphs and thus, although the approximated mean differed substantially from that measured, this difference was not statistically significant (Table 2). Overall, the effect of this source of error is less than might initially be expected as this factor occurs in both the numerator and denominator of equation (1). A simple and effective means of minimizing this source of error would be to collect gut contents, by either of the above methods, and measure the weight loss during digestion. Sediment from the animal's surroundings could be used if gut contents cannot be obtained. The mean percentage weight loss could then be used to correct the weights of digested gut contents.

These two sources of error may either counteract one another, yielding corrected values that are not significantly different from those measured directly (As, Cu), or be additive, resulting in corrected values that are significantly larger than the actual measures (Cd, Zn) (Table 2, c). However, since these sources of error may be minimized: (a) by measuring trace element burdens of gut contents, and (b) by compensating for weight loss of gut contents during digestion, the correction method could provide good estimates of body pollutant burdens.

Conclusions

The precise measure of pollutant concentrations in the bodies of benthic animals is usually confounded by the presence of contaminated gut contents. Their contribution to body burdens may in some cases be ignored if: (a) gut content pollutant levels are much lower than those of the animal, (b) a crude estimate is required (the cost of analyses may not warrant crude measures), and (c) one is studying the transfer of pollutants from prey to predators that consume their prey in their entirety, i.e. including gut contents.

In order to obtain precise estimates of pollutant body burdens it is usually necessary to remove or somehow compensate for the pollutants present in an animal's gut contents. Three possible approaches have been compared: (i) direct removal of the gut contents by dissection; (ii) induced removal of the gut contents (depuration); (iii) analysis of the whole animal (including gut contents), followed by a correction for the contribution afforded by gut contents. Method (i) provides measures that are precise and unambiguous and, where feasible, is the technique of choice for *H. limbata*. Method (ii) is a possible alternative, but the effects of the necessarily lengthy depuration period in water (48 h or more) on trace element levels of the organism are presently unknown. Depuration in uncontaminated sediments is a better method, provided that suitable sediments can be found (or manufactured). Method (iii) offers simplicity and savings in time; despite the inherent biases regarding the weight and trace element levels of the gut contents (which may be minimized, as discussed above), it yielded reasonably accurate estimates (max. error <25%) of the trace element content of *H. limbata* in the present study. In the final analysis, the choice of method will depend on the goals of and means available to an investigator.

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Among-Population Variability of Fish Growth: I. Influence of the Quantity of Food Consumed[1]

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We assessed the relationship between in situ fish growth and food consumption for a series of yellow perch (*Perca flavescens*) populations that exhibited a 1.8 to 10-fold range in growth rates on a weight basis. Perch from 12 populations (lakes) and three year–classes (I+, II+, and III+) were sampled at 3–4 wk intervals from late May to late September 1985. On each occasion, water temperature profile and daily ration were determined. Among-lake water temperature differences were minimal (of the 12 lakes studied 10 had mean water temperatures of 19.0 ± 1°C) and could not explain growth differences. Food consumption covered a four-fold range within each age–class and varied significantly among populations but not among age–classes. Food consumption explained a significant proportion of among-population variability in perch growth rates only for age I+ fish ($r^2 = 0.52$). We argue that other factors such as prey type, prey availability, and fish community descriptors are important determinants of among-population growth variability in the lakes we studied, and by implication, in other systems.

Nous avons évalué la relation entre la croissance *in situ* des poissons et la quantité de nourriture consommée pour un ensemble de populations de perchaudes (*Perca flavescens*) dont le taux de croissance en poids varie par un facteur de 1.8 à 10. Des perchaudes de 12 populations (lacs) et trois classes d'âge (I+, II+, et III+) furent échantillonnées à un intervalle de 3–4 sem de la fin Mai à la fin Septembre 1985. A chaque occasion, le profil de la température de l'eau et la ration journalière furent déterminés. Les variations inter-lacs de la température de l'eau furent minimes (des 12 lacs étudiés 10 ont eu des températures moyennes de 19.0 ±1°C) et n'ont pas expliqué les variations de la croissance. La quantité de nourriture consommée a varié par un facteur de quatre à l'intérieur de chaque classe d'âge et a varié significativement entre les populations mais pas entre les classes d'âge. La quantité de nourriture consommée n'a expliqué une proportion significative de la variabilité inter-population du taux de croissance que pour des perchaudes d'âge I+ ($r^2 = 0.52$). Nous soutenons que d'autres facteurs tels le type de proies, la disponibilité des proies et des descripteurs de la communauté de poissons sont des d'importants déterminants des variations inter-populations de la croissance pour les lacs étudiés, et par extension, pour d'autres systèmes.

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This paper is the first of an intended series of three in which we assess the importance of food consumption, prey type, and fish community descriptors as regulators of among-population variability in in situ fish growth.

Since the Winberg (1958) description of fish growth as a balanced energy equation, it has been implicitly accepted that the growth of fish in natural populations is strongly regulated by the quantity of food consumed. Laboratory studies have supported this view by demonstrating a positive relationship between food consumption and growth for many fish species (see Paloheimo and Dickie 1966; Brett and Groves 1979; Elliott 1979; Condrey 1982; and Soofiani and Hawkins 1985 for reviews). Laboratory studies have also demonstrated positive relationships between food consumption and physiological processes such as specific dynamic action (Kleiber 1961; Muir and Niimi 1972; Beamish 1974; Jobling 1983b), and urinary and fecal loss (Weisberg and Lotrich 1982; Elliott 1976b; Solomon and Brafield 1972; Pandian 1967; Soofiani 1983). These findings have reinforced the view that food consumption is a major regulator of fish growth in situ.

The form of the relationship between fish growth and food consumption has theoretical and practical implications. For example, in situ food consumption has been estimated from a knowledge of in situ growth rates and laboratory derived values of growth efficiency (Brocksen et al. 1968; Davis and Warren 1971; Carline et al. 1973; Elliott 1975; Allen and Wootton 1982; Pauly 1986). The strength of the laboratory derived relationship between growth and consumption has also led to inferences about the causes of among-population, among-site, or among-year variability in fish growth. The need for these indirect approaches has been dictated by the paucity of reliable estimates of in situ food consumption (Brocksen et al. 1970; Walline 1985; Allen and Wooton 1982; Naesje et al. 1987; Weatherley 1987). The experimental evidence for a relationship between growth rates and food consumption has also served as

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a motivation for large-scale lake fertilization programs (Hall et al. 1970; Hyatt and Stockner 1985; Mills 1985) intended to enhance fish growth and production.

It has long been recognized, however, that the extrapolation of laboratory derived relationships between growth and food consumption to natural situations depends heavily on the extent to which the experimental conditions adequately reflect fish diet, physiological condition, activity level, water temperatures, and food availability. These conditions are, of course, never completely met (Mann 1978).

In this paper we have sought to directly assess the importance of differences in the quantity of food consumed as a regulator of among-population variability in in situ fish growth.

Methods

Species and Populations Studied

The species chosen for study was the yellow perch (*Perca flavescens*). Yellow perch are known to exhibit high variability in growth rates within restricted geographic areas (Alm 1946; Grimaldi and Leduc 1973; Hansson 1985). This permits the study of growth in populations that experience similar natural water temperature regimes, an important consideration since temperature is known to significantly influence growth rates (Hile 1941; LeCren 1958; Elliott 1976a; Jobling 1983a; Henderson and Brown 1985; Cuenco et al. 1985).

Preliminary sampling of lakes of the Eastern Townships region of Quebec (Fig. 1) during 1984 identified 12 separate populations exhibiting a range of growth rates (Fig. 2) that approached the full range reported for perch at this latitude (Grimaldi and Leduc 1973; Nakashima and Leggett 1975; Heath and Roff 1987). The morphological and limnological characteristics of the lakes surveyed are described in Rasmussen (1988a,b).

Sampling

Each population was sampled once every 3–4 wk between late May and late September of 1985. This interval encompassed the growing season for perch at this latitude (Jobes 1952; El-Zarka 1959; Coble 1965; Fortin and Magnin 1972; Nakashima and Leggett 1975). On each occasion we collected 40 perch ranging from 50–150 mm total length (TL). Ten fish were collected every 8 h over a period of 24 h beginning at 10:00. All fish were collected with a 50 m × 3 m (0.6 cm^2-mesh) knotless nylon bag seine deployed from shore in the littoral zone. Fish were killed with an overdose of 2-phenoxyethanol (1 $ml \cdot L^{-1}$) immediately following capture. The complete digestive tract contents of each fish were removed and weighed individually in the field (± 0.01 g wet weight) within 3 h of capture. Fish total length (± 1 mm) and weight (± 0.1 g wet weight; minus digestive tract content) were determined on site. Ten to 20 scales were collected from each fish for aging. While problems of aging perch from scales can occur (Craig 1987), they generally occur when fish older than 5 yr are assessed. Sex was noted. The stomach contents of 10 fish from each sampling session were dried to constant weight at 60°C on site and individually used to estimate the caloric value of the diet.

Determination of Water Temperature

We selected our sampling sites to minimize the influence of temperature on our analyses. The mean surface temperature (0–5 m) was determined by measuring water temperatures at 1-m intervals during each sampling session at each lake. The 5-m depth was chosen because it incorporates the habitat of most perch of the size spectrum we studied (Wells 1968; Werner et al. 1977; Keast et al. 1978) and conformed to the depth sampled by our seine. We used seasonal mean water temperature (SMWT) as an integrated measure of lake water temperatures experienced by the perch populations we studied. SMWT was

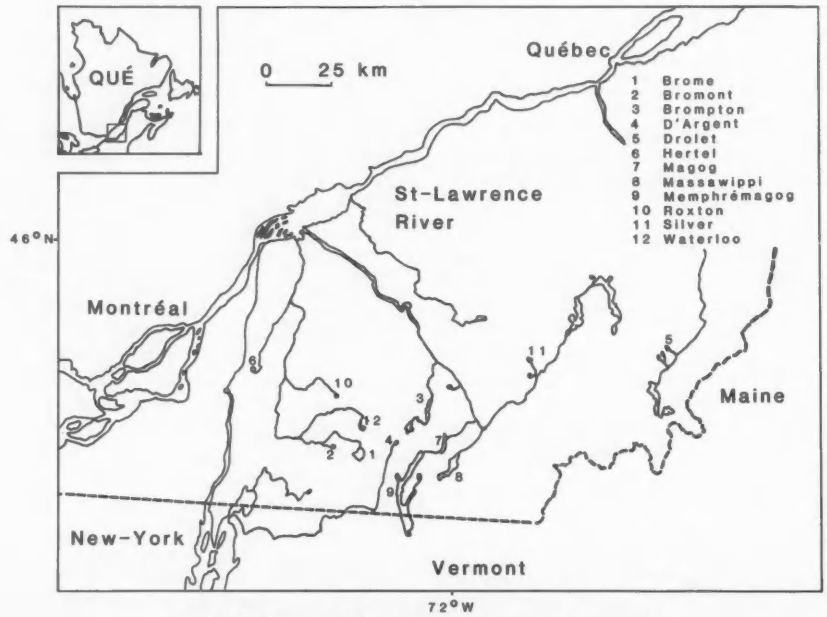

FIG. 1. Study area and the location of the populations sampled.

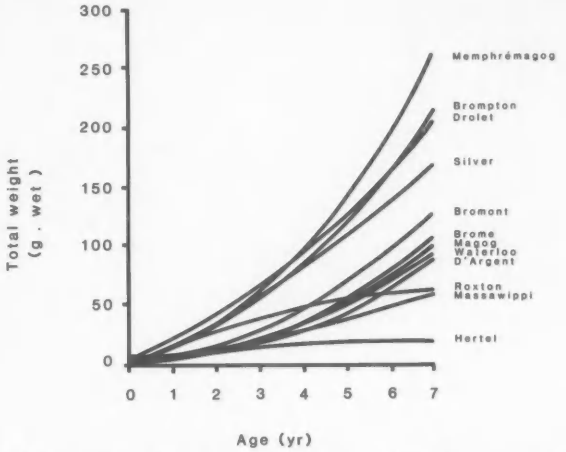

FIG. 2. Weight at age relationships for the perch populations studied.

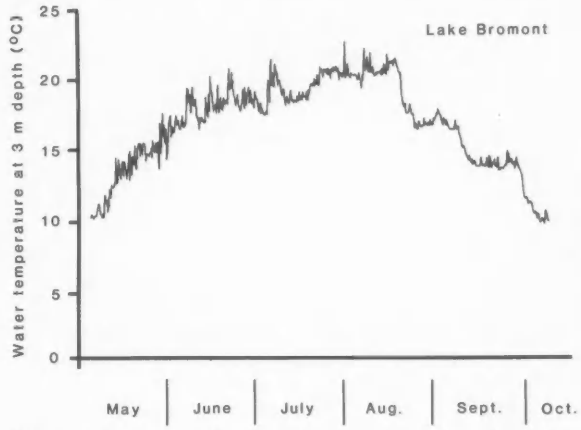

FIG. 3. Time series of water temperature at 3-m depth in Lake Bromont.

estimated for each lake by dividing cumulative degree days (the area under the curve expressing the relationship between mean water temperature in the 0–5 m depth level and Julian days) by the number of days between the first and last fish sample collected from that lake.

We assessed the effect of sampling frequency on estimates of SMWT by analyzing a continuous water temperature record obtained with a Ryan thermometer moored in Lake Bromont (Fig. 3) from May to October at a depth of 3 m on the 5-m isobath. From this 163-d temperature record we extracted 650 independent water temperature measures taken at 6-h intervals. We then calculated eight SMWT values by varying the sampling interval through 0.25, 0.50, 1, 2, 4, 8, 16, and 32 d. There was no effect of sampling frequency on estimated SMWT. Water temperatures sampled at 0.25-d intervals yielded a SMWT of 16.4°C compared with estimates of 16.3 and 16.6°C derived from sampling intervals of 8 and 32 d, respectively. To preclude the possibility that these sampling frequencies yielded similar SMWT values by chance, a second simulation was performed. SMWT was estimated from 6-h periods using a sampling frequency of 6 h and 32 d. This procedure was repeated 127 times with the starting point being successively varied from

TABLE 1. Sampling interval (SI), mean fish weight (W_m) for each combination of perch age–class and population studied, and seasonal mean water temperature (SMWT) for each lake surveyed.

Population	SI (d)	W_m (g wet weight) I+	II+	III+	SMWT (°C)
Brome	76			24.4	19.8
Bromont	98	6.2	15.8	26.8	21.2
Brompton	74	5.9	16.4	26.8	19.7
D'Argent	112	8.0	16.7	24.1	19.2
Drolet	90	14.3		34.8	18.1
Hertel	97		6.8	10.1	19.4
Magog	78		19.3	31.8	19.9
Massawippi	75	5.3	10.6		19.6
Memphremagog	79	7.4	17.4	26.4	19.3
Roxton	98		10.7	18.1	21.3
Silver	91	8.9	33.0		18.9
Waterloo	85	7.0	16.8	23.0	19.8

TABLE 2. Absolute growth rate (AGR) and absolute daily ration (ADR) for each combination of perch age–class and population studied.

Population	AGR (g wet weight · d^{-1}) I+	II+	III+	ADR (g dry weight · d^{-1}) I+	II+	III+
Brome			0.05			0.32
Bromont	0.05	0.04	0.04	0.02	0.07	0.11
Brompton	0.06	0.11	0.08	0.05	0.15	0.26
D'Argent	0.07	0.02	0.05	0.04	0.09	0.13
Drolet	0.19		0.10	0.13		0.31
Hertel		0.02	0.02		0.07	0.10
Magog		0.18	0.11		0.19	0.35
Massawippi	0.07	0.02		0.07	0.14	
Memphremagog	0.11	0.13	0.12	0.06	0.15	0.21
Roxton		0.06	0.07		0.08	0.15
Silver	0.12	0.33		0.08	0.30	
Waterloo	0.07	0.06	0.14	0.02	0.05	0.07

day 1 to day 127. For each run we calculated the difference between the two estimates of SMWT derived using the 6-h and 32-d frequency. The mean difference between SMWT values estimated from 6-h and 32-d sampling intervals was <0.1°C ($n = 127$; $s = 0.41$) and did not differ significantly from 0 ($t_{126} = 0.002$; $p > 0.9$). We conclude that the 21–28 d sampling interval we employed yielded precise estimates of SMWT in the lakes studied.

Field Evaluation of the Relationship between Growth and Consumption

Verification of a significant positive relationship between fish growth and food consumption requires coincident estimates of increases in body weight, and of the quantity of food consumed. The best design would involve analysis of fish of similar initial sizes sampled over equal time intervals. Unfortunately, field studies are seldom amenable to this straightforward approach.

In our study unavoidable between-lake variability in sampling interval (Table 1), required that growth and food consumption be expressed and analysed as rates.

Estimation of Growth Rates

Growth rate was estimated for each of three age–classes (I+, II+, III+) in each population. We estimated the absolute

TABLE 3. Statistics of the linear functions relating absolute growth and consumption rates to mean fish weight for each age–class studied.

	F	df	p	r^2
Absolute growth rates				
I+	39.53	1,6	<0.001	0.87
II+	33.75	1,8	<0.005	0.81
III+	5.32	1,8	<0.05	0.40
Absolute consumption rates				
I+	9.09	1,6	<0.03	0.60
II+	13.09	1,8	<0.01	0.62
III+	5.61	1,8	<0.05	0.41

TABLE 4. Statistics of the among-population and among- age–class variability in mean fish weight, growth, and consumption rates (two-way ANOVA).

	F	df	p
Mean fish weight			
population	99.67	11,746	<0.0001
age-class	810.89	2,746	<0.0001
interaction	8.88	14,746	<0.0001
Absolute growth rates			
population	18.44	11,101	<0.0001
age-class	3.44	2,101	<0.05
interaction	4.40	14,101	<0.0001
Absolute consumption rates			
population	100.59	11,852	<0.0001
age-class	327.24	2,852	<0.0001
interaction	2.56	14,852	<0.002
Relative growth rates			
population	4.14	11,101	<0.0001
age-class	48.92	2,101	<0.0001
interaction	1.35	14,101	>0.15
Relative consumption rates			
population	66.23	11,852	<0.0001
age-class	0.01	2,852	>0.90
interaction	0.01	14,852	>0.90

TABLE 5. Statistics of the among-population variability in mean fish weight, growth, and consumption rates for each age–class studied (one-way ANOVA).

	F	df	p
Mean fish weight			
I+	22.20	7,266	<0.0001
II+	66.11	9,265	<0.0001
III+	55.33	9,215	<0.0001
Absolute growth rates			
I+	16.68	7,42	<0.0001
II+	16.22	9,35	<0.0001
III+	5.04	9,24	<0.0001
Absolute consumption rates			
I+	42.31	7,235	<0.0001
II+	45.84	9,305	<0.0001
III+	48.39	9,312	<0.0001
Relative growth rates			
I+	4.67	7,42	<0.001
II+	3.64	9,35	<0.005
III+	2.64	9,24	<0.03
Relative consumption rates			
I+	30.69	7,235	<0.0001
II+	27.35	9,305	<0.0001
III+	29.77	9,312	<0.001

growth rate (AGR; g wet weight·d^{-1}) for each combination of perch age–class and population using equation 1,

$$(1)\quad \mathrm{AGR} = \frac{W_1 - W_f}{J_1 - J_f}$$

where W_f and W_l are respectively mean fish weights at the first (J_f) and last (J_l) Julian date of the sampling season.

The mean weight of fish in each age–class (Table 1) varied significantly among populations and age–classes (Table 4) and also among populations for each age–class (Table 5). Furthermore AGR was significantly and positively correlated with mean fish weight within all age–classes examined (Table 3). For this reason we have chosen to base our analyses on relative growth rates.

We estimated relative growth rates (RGR; growth rates weighted for fish size; g wet weight·g wet weight^{-1}·d^{-1}) for each combination of perch age–class and population using equation 2,

$$(2)\quad \mathrm{RGR} = \frac{\mathrm{AGR}}{W_m}$$

where W_m is the mean fish weight during the interval $J_l - J_f$.

Estimation of Consumption Rates

It is important that the function best describing food evacuation be employed in models used to estimate daily ration (Pennington 1985; Olson and Mullen 1986; Persson 1986; Jobling 1986). Food evacuation in perch has been repeatedly shown to follow an exponential function (Persson 1979; 1981; Nakashima 1979; Boisclair and Leggett 1985, 1988). We used the Eggers (1977) model, which assumes an exponential evacuation function, to estimate the average daily ration of all fish on a given date i (D_i; g dry weight·d^{-1}). The efficiency of this model for studies of food consumption involving perch has been previously established (Boisclair and Leggett 1988). The Eggers model has the form,

$$(3)\quad D_i = F_i \times R_i \times 24$$

where F_i is the mean gut fullness (total digestive tract content:g dry weight) and R_i is the evacuation rate (h^{-1}; Elliott 1972; Kitchell et al. 1978; Grove and Crawford 1980; Persson 1982) on sampling date i. Mean gut fullness and evacuation rate were estimated on site for each combination of perch age–class and population on each sampling date. The methodology used to estimate both R_i and the standard error about D_i are detailed in Boisclair and Leggett (1988). A seasonally integrated measure of food consumption was estimated by dividing the total quantity of food consumed per individual of a given combination of perch age–class and population (area under the relationship between D_i and Julian days) by the number of days between the first and last sampling date for that population. This quantity is hereafter referred to as absolute daily ration (ADR; g dry weight·d^{-1}).

ADR was significantly and positively correlated with mean fish weight within all age–classes examined (Table 3). For this reason we have chosen to base our analyses on relative daily ration estimates.

We estimated relative daily ration (RDR; daily ration weighted for fish size; g dry weight·g wet weight^{-1}·d^{-1}) for each combination of perch age–class and population using equation 4,

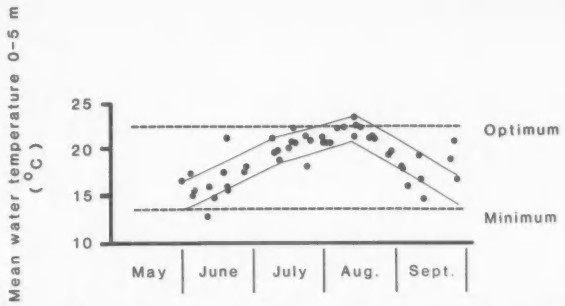

FIG. 4. Mean water temperature from surface to 5-m depth in the 12 lakes surveyed. Broken lines represent minimum and optimum water temperatures for perch growth. The 3°C band includes 80% of the mean water temperatures determined.

$$(4)\quad \mathrm{RDR} = \frac{\mathrm{ADR}}{W_m}$$

where W_m is the mean fish weight during the interval $J_i - J_f$.

Estimation of Growth Efficiency

Gross growth efficiency (K_i ; percent) was calculated for each combination of each age–class and population using equation 5 (Ivlev 1939),

$$(5)\quad K_i = \frac{\mathrm{AGR}}{\mathrm{ADR} \cdot 6.97} \times 100$$

where 6.97 is the average ratio of wet to dry weight of gut contents as determined by this study.

Estimation of Caloric Value of the Diet

For each sampling session at each lake 10 stomach contents were subjected for bomb calorimetry to determine the caloric contents of the food consumed (total calories·g dry weight^{-1}). A Phillipson microbomb calorimeter (Paine 1971) was used in these analyses. Each sample was burned once.

Relationship between Growth and the Quantity of Food Consumed

The relationship between growth rates (or growth efficiency) and the quantity of food consumed was assessed independently for each perch age–class to ensure that the data analyzed as replicates of a given event (in our case growth rates or efficiency among populations) are statistically independent. Our observations indicate that perch in the populations we studied attain their sexual maturity at the age of III+. Separate analysis of these age–classes thus ensured that the patterns observed were not the result of among age–class differences in energy allocation or ontogenetic changes in physiological constraints.

Results

Water Temperature

As expected the water temperature regimes of the 12 lakes surveyed were very similar. Approximately 80% of the variation in the mean (surface to 5 m) water temperatures recorded among the 12 lakes on any given sampling session was subsumed by a 3.0°C band about the mean (Fig. 4). The lowest and highest mean water temperatures observed were respectively within 1°C of the minimum (13.5°C; LeCren 1958; Nakashima and Leggett 1975) and optimum (22.5°C; Schneider 1973; Neil and Magnuson 1974) water temperatures for perch growth (Fig. 4). The seasonal mean water temperature (SMWT) ranged from 18.1 to 21.3°C (Table 1). Of the 12 lakes studied 10 had a SMWT of 19.0 ± 1°C. There was no statistical relationship between relative growth rates or growth efficiency and SMWT in the populations studied ($p > 0.05$). The addition of food consumption estimates to the list of independent variables did not alter this interpretation ($p > 0.05$).

TABLE 6. Statistics of the relationships between growth and food consumption for the three age–classes studied (AGR, RGR, and K_1 are respectively the absolute and relative growth rates and the growth efficiency; ADR and RDR are respectively the absolute and relative daily ration). Degrees of freedom associated with F values are 1,6 for age I+ fish and 1,8 for age II+ and III+ fish.

	F	p	r^2
AGR versus ADR			
I+	24.04	<0.003	0.80
II+	40.95	<0.0002	0.84
III+	0.89	>0.30	
RGR versus RDR			
I+	6.52	<0.05	0.52
II+	0.61	>0.40	
III+	0.68	>0.40	
K_1 versus ADR			
I+	2.55	>0.15	
II+	1.32	>0.25	
III+	2.09	>0.15	

Growth

Covariance analyses performed on each combination of perch age–class and population indicated that sex did not contribute significantly to differences in the growth rates observed ($p > 0.05$). This result is consistent with previous findings for perch (Ney and Smith 1975; Nakashima and Leggett 1975; Hansson 1985). We therefore pooled sexes when estimating growth rates in all subsequent analyses. These pooled data were sufficient to permit analysis of the growth rates of I+ fish from eight populations, and II+ and III+ fish from 10 populations.

Absolute growth rates (AGR: g wet weight·d^{-1}) of I+ perch varied 3.5-fold among populations. Corresponding values for II+ and III+ perch were 16.8-fold and 6.5-fold, respectively (Table 2). Absolute growth rates varied significantly both among populations and among age–classes (two-way ANOVA; Table 4). Among-population differences in absolute growth rates were also significant when each age–class was analysed separately (one-way ANOVA; Table 5).

Relative growth rates (RGR; g wet weight·g wet weight^{-1}·d^{-1}) of I+ perch varied 1.8-fold among populations. Corresponding values for age II+ and III+ perch were 10-fold and 3-fold, respectively (Table 2). These differences in relative growth rates were significantly both among populations and among age–classes (two-way ANOVA; Table 4). Among-population differences in relative growth rates were also significant when each age-class was analysed separately (one-way ANOVA; Table 5).

Quantity of Food Consumed

Absolute daily ration (ADR; g dry weight·d^{-1}) of age I+ perch varied 6.1-fold among populations. Corresponding val-

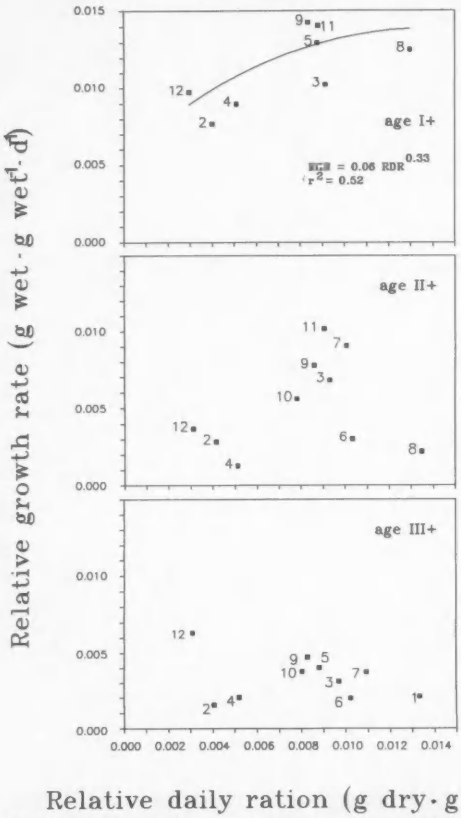

FIG. 5. Relationship between relative growth rates (RGR) and relative daily ration (RDR) for the 12 perch populations identified in Fig. 1.

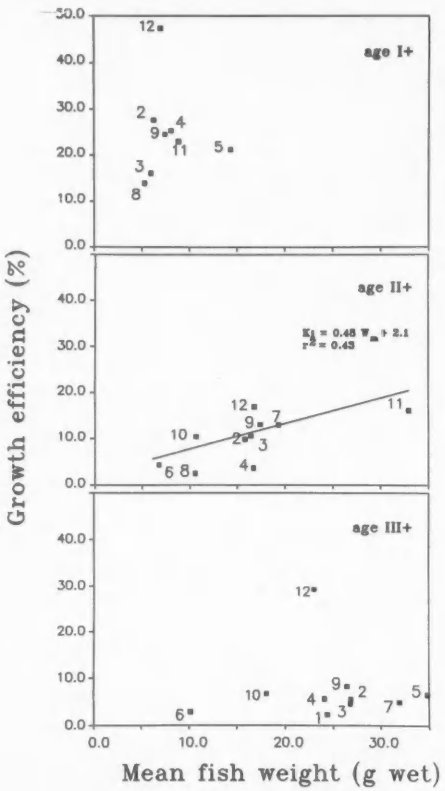

FIG. 6. Relationship between growth efficiency (K_1) and mean fish weight (W_m) for the 12 perch populations identified in Fig. 1

ues for II+ and III+ perch were 3.8- and 4.9-fold, respectively (Table 2). These differences in absolute daily ration were significant both among populations and among age–classes (two-way ANOVA; Table 4). Among-population differences in absolute daily ration were also significant when each age–class was analysed separately (one-way ANOVA; Table 5).

Relative daily ration (RDR: g dry weight·g wet weight^{-1}·d^{-1}) varied four-fold among populations and ranged from 0.003 (Lake Waterloo: age III+) to 0.013 (Lake Massawippi: age II+). Relative daily ration varied significantly among population but not among age–classes (two-way ANOVA; Table 4). Among-population differences in relative daily ration were also significant when each age–class was analysed separately (one-way ANOVA; Table 5).

Relationship between Growth Rates and Food Consumption

We found a significant relationship between AGR and ADR for age I+ and II+ fish (Table 6). However, because both AGR and ADR were strongly related to fish size we used RGR and RDR as variates (Fig. 5). Only age I+ perch exhibited a significant relationship between RGR and RDR (Table 6). Application of a correction for bias in log-transformed equations (Sprugel 1983) did not alter the coefficients of the relationship between RGR and RDR for age I+ fish.

Growth Efficiency

Growth efficiencies estimated from our data ranged from 13.9 (Lake Massawippi) to 47.3% (Lake Waterloo) for age I+ fish, 2.3 (Lake Massawippi) to 16.8% (Lake Waterloo) for age II+ fish, and from 2.24 to 29.2% (Lake Waterloo) for age III+ fish. These values varied significantly among populations ($F_{11,14} = 5.17$; $p < 0.005$) and age-classes ($F_{2,14} = 27.79$; $p < 0.0001$). Growth efficiencies reported in the literature generally range from 0 to 20% and decrease with fish size or age (Paloheimo and Dickie 1966; Brett and Groves 1979; Ware 1982). These comparisons suggest that our estimates of growth efficiency for Lake Waterloo are suspiciously high. Our analyses of growth efficiency were thus executed both including and excluding values from that lake.

Relationship between Growth Efficiency, Fish Weight, and Daily Ration

The results of laboratory studies indicate that individual growth efficiency increases with fish size and feeding level at low values of either, and decreases with fish size and ration at high values (Paloheimo and Dickie 1966; Kerr 1971; Brett and Groves 1979; Ware 1982). Our data reveal a tendency for growth efficiency to increase with fish weight in both age I+ and II+ perch (Fig. 6). However the relationship was significant only for age II+ perch (age I+: $F_{1,6} = 0.003$; $p > 0.95$; age II+:

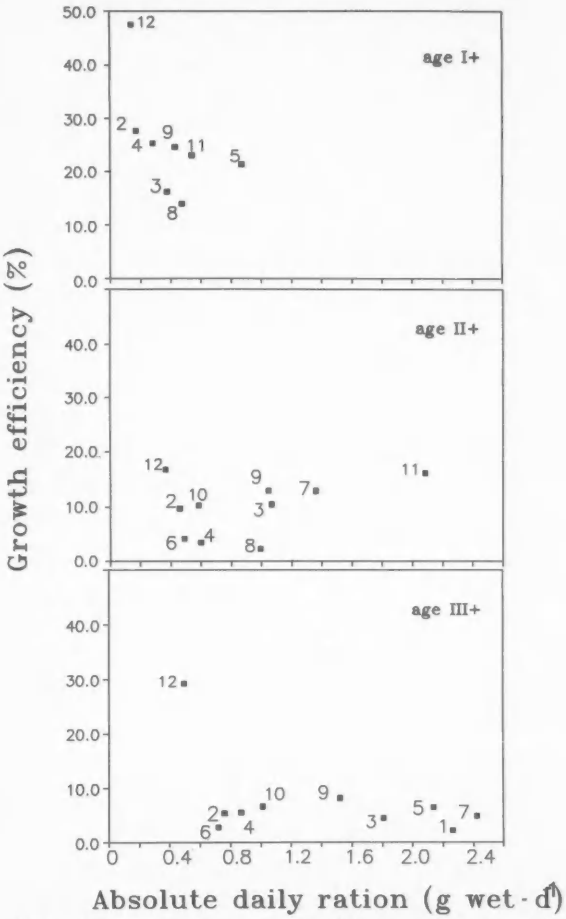

FIG. 7. Relationship between growth efficiency and absolute daily ration for the 12 perch populations identified in Fig. 1.

$F_{1,8} = 6.06$; $p < 0.05$; age III+: $F_{1,8} < 0.001$; $p > 0.95$). Analyses of these relationships using the logarithm of K_1 (Paloheimo and Dickie 1966) did not alter our interpretation. Exclusion of the Lake Waterloo values from the analysis did not alter the pattern in the data.

We found no significant relationship between growth efficiency and absolute ration levels within age–classes (Table 6). Neither the use of the logarithm of K_1, nor the exclusion of Lake Waterloo from our analysis influenced the significance of the relationships. However, these treatments did reveal very different trends in the data set (Fig. 7). When Lake Waterloo was excluded, K_1 showed a tendency to decrease with ration level for age I+ fish, and increase with ration level for age II+ fish. For age III+ fish, the trajectory of K_1 was parabolic. While these patterns are all compatible with the hypothesis of a relationship between K_1 and fish feeding levels, the diversity of the trends presented by our data and the scatter observed precludes a general conclusion.

Caloric Content of the Diet

The grand mean caloric value of all stomach contents analyzed was 4417 cal·g^{-1} (dry weight). The caloric value of food consumed did not vary significantly either among populations ($F_{11,130} = 0.54$; $p > 0.80$) or among age–classes ($F_{2,130} = 1.32$; $p > 0.20$). It did, however, vary significantly among sampling dates ($F_{3,130} = 5.94$; $p < 0.001$). Most of the interaction terms examined were significant (date × population: $F_{33,130} = 3.29$; $p < 0.0001$; date × age: $F_{6,130} = 2.93$; $p < 0.01$; population × age: $F_{21,130} = 1.81$; $p < 0.05$; date × population × age: $F_{26,130} = 0.86$; $p > 0.60$). We concluded that the observed among-population variability in fish growth rates was not related to differences in food caloric content.

Discussion

Our analyses indicate that food consumption did not consistently explain a significant fraction of the among-population variability of fish growth in the populations we studied despite the experimental framework supporting the existence of a strong relationship between growth and food consumption. Only one of the three perch age–classes exhibited a significant positive relationship between fish growth rates and the quantity of food consumed.

We are aware of only one other study that attempted to explain in situ growth rates differences using field derived estimates of food consumption. Hayward and Margraf (1987) argued that perch of the central basin of Lake Erie grew faster than those of the western basin because of higher food consumption rates. The strength of their conclusion is weakened by three factors. First, they are limited by the number of situations studied (n = 2). Second, the food consumption estimates used were derived from models in which evacuation rates were predicted from water temperature. They thus neglected the influence of food type on evacuation rate, a very important source of error since diet differences between basins were dramatic. Third, and most important, their data do not support their interpretation of the between-basin growth differences. From their figure 9, we estimated that perch absolute growth rates (g wet weight·d^{-1}) for the primary sampling sites in the western (Middle sister) and central (Fairport) basins were 0.07 and 0.17 (age I+), 0.18 and 0.22 (age II+), and 0.08 and 0.35 (age III+), respectively. In all cases, however, perch were larger in the central than in the western basin. It has long been recognized that larger fish tend to grow faster than smaller ones (Parker and Larkin 1959; Ricker 1979). As our data confirm, perch growth rate is a positive linear function of fish size, even within an age–class.

We thus determined the relative growth rates (g wet weight·g wet weight^{-1}·d^{-1}) of perch at the primary sampling sites in the western and central basins of Lake Erie to be 0.007 and 0.005 (age I+), 0.006 and 0.003 (age II+), and 0.002 and 0.004 (age III+), respectively. Hence, when corrected for fish size, between-basin growth differences were reversed for age I+ and II+ fish relative to the interpretation of Hayward and Margraf (1987).

We conclude that the growth differences noted by these authors for age I+ and II+ perch result from size differences. Age III+ perch do grow faster in the central than in the western basin of Lake Erie. Unfortunately, Hayward and Margraf did not provide the data required to estimate RDR for these fish.

We acknowledge that our evaluation of their data are subject to errors related to the extraction of data from a figure. However, these analyses do demonstrate that until a more precise treatment of their results is available, their conclusions should be accepted with caution.

The possibility that the growth differences we observed were due to genetic differences (Allendorf and Utter 1979; Kirpichnikov 1981) was investigated for growth differences in I+ perch from lakes Memphremagog and Hertel (two extremes of growth rates in the populations we studied). Individuals from these two populations had identical growth rates when reared together in identical laboratory conditions (Heath and Roff 1987). Similar results were obtained when perch (Alm 1946), northern pike, *Esox lucius* (Carbine 1944), and brook trout, *Salvelinus fontinalis* (Reimers 1979) from stunted populations were reared under laboratory conditions. We therefore consider it unlikely that genetic differences regulated growth in the populations we studied.

The fact that food consumption fails to adequately explain interpopulation differences in in situ growth rates even when differences in both food consumption and growth are very large suggests that these differences may result from differences in growth efficiency (the portion of food consumption expressed as growth). The exponential decrease of growth efficiency with ration level (K-line phenomenon: Paloheimo and Dickie 1966) has received both support (Kerr 1971; Brett and Groves 1979) and criticism (Condrey 1982). At best, our data suggest the existence of a weak in situ relationship between K_1 and ration level within age–classes. However, the shape of this relationship is indeterminate. The weakness of the K-lines we derived is not surprising. Growth efficiency is generally believed to depend on prey quality (e.g. species, size, caloric content), prey availability, and on the interactions between fish (Paloheimo and Dickie 1966; Kerr 1971; Ware 1972). The populations we studied differed dramatically both in diet and the composition of the fish community of which they were part (Boisclair and Leggett 1989a,b). Although many laboratory experiments have described the influence of caloric content of the food on fish growth (Phillips and Brockway 1959; Ringrose 1971; Brett 1976a,b; Craig and Fletcher 1984), the lack of among-population variability in the caloric content of the food consumed by the perch we studied suggests that this component of prey quality had a minor influence on growth of these populations.

Prey quality (e.g. species or size), prey availability, and fish community (e.g. species, biomass, numerical density) have been hypothesized to affect growth efficiency via their influence on the quantity of energy expended for activity metabolism (Paloheimo and Dickie 1966; Kerr and Martin 1970; Wankowski and Thorpe 1979; Werner 1979; Hart and Connellan 1984). Activity metabolism, as influenced by behavior associated with feeding, may be the most important physiological component of the bioenergetics models developed to explain among-population variability in fish growth (but see Rice et al. 1983). This could also explain the discrepancy between the seasonal mean growth rates we observed and those predicted by Kitchell et al. (1977). The laboratory based bioenergetics model they developed predicts that the relative growth rates of a 10-g fish at 20°C should range between 0.015 and 0.070 g wet weight·g wet weight^{-1}·d^{-1}. Growth rates of perch of equivalent mean wet weights (age II+: Lake Massawippi, 10.6 g wet weight; Lake Roxton, 10.7 g wet weight; age III+: Lake Hertel, 10.1 g wet weight; Table 1) exposed to similar temperatures (SMWT: Lake Massawippi; 19.6; Lake Roxton; 21.3 Lake Hertel; 19.4°C; Table 1) were respectively 0.002, 0.006, and 0.002 g wet weight·g wet weight^{-1}·d^{-1}. The growth rates we observed for 10-g perch at approximately 20°C were thus two to seven-fold lower than the lowest growth rates predicted by the Kitchell et al. (1977) model. In their model Kitchell et al. (1977) assumed constant active metabolism equal to twice the standard metabolic rate.

Many different ratios of active metabolism/standard metabolic rate have been employed in energetics analyses. Winberg (1956) suggested that a value of 2 was applicable for most species, but values ranging from 0.2 to 1.5 have been used for largemouth bass (*Micropterus salmoides*) (Adams et al. 1982) depending on the time of year; Diana (1983, 1987) used a value of 1 for northern pike *Esox lucius*; Kerr (1971) used values ranging from 1–3 for lake trout *Salvelinus namaycush* and from 1–4.5 for cod *Gadus morhua* (Kerr 1982) and Brett and Groves (1979) used a value of 8 for migrating sockeye salmon *Oncorhynchus nerka*. All of these ratios were derived from laboratory experiments.

While water temperature, fish diet, and physiological condition can be simulated in laboratory, other parameters such as food availability and within- or among-species interactions (predatory risk, competition, etc.) are not adequately represented in most laboratory protocols (Mann 1978; Kerr 1982).

The quantity of energy expended for activity is generally considered equal to that deposited as growth (approximately 20% of energy ingested; Brett and Groves 1979; Ware 1982; Adams et al. 1982). If so, differences in activity level resulting from differences in prey quality, prey availability, and/or interference from other fish could alter growth efficiency from 0% (for fish so active that no energy is left for growth) to 40% (for fish with no activity expenditure). Although these limits are approximate and depend on environmental conditions, among-population differences in activity level have the potential to explain the growth differences observed in our study.

The sensitivity of bioenergetics models to variations in parameters concerning food consumption and activity (Kitchell et al. 1977; Bartell et al. 1986) further emphasizes the need for quantitative consideration of activity levels in these analyses. Quantifying the energy allocated to activity is, unfortunately, extremely difficult in situ (see Soofiani and Hawkins 1985 for a review). While the use of swimming speed/water temperature relationships has been successfully used to account for activity in energetics modeling (Rice et al. 1983; Stewart and Binkowski 1986) these relationships do not consider the potential influence of prey type and fish interactions on activity and growth. The influence of activity on growth in situ can, however, be approached indirectly through an analysis of the influence of prey type, prey biomass, and fish community characteristics that are more easily studied. The relationships between fish growth, prey type, and fish community descriptors are examined in subsequent papers (Boisclair and Leggett 1989a,b). These relationships indicate that fish community descriptors, presumably fish numerical density (all species combined), are the major regulators of among-population variability in growth in the 12 populations we studied. The influence of prey availability on their growth is presently under analysis.

The inability of observed differences in food consumption to adequately account for among-population differences in the growth rates of the fish we studied has important theoretical and practical implications. Many field studies have revealed significant negative relationships between fish population density and growth (Peterman 1984; Zalewski et al. 1985; and Backiel and LeCren 1978; and Brett 1979 for reviews). Although these field studies have not been supplemented with food consumption estimates, the most common interpretation of that finding has been that fish density influenced fish growth via a decrease in the average quantity of food consumed. Our

analyses suggest that caution should be applied to this interpretation until adequate data are obtained.

Large-scale lake fertilization programs appear to be based on the premise that increasing prey abundance will increase fish production. While early results have been encouraging (Hall et al. 1970; Hyatt and Stockner 1985; Mills 1985), these studies have rarely exceeded a time period of 10 yr. It is not known whether this time scale is sufficient to determine if increases in fish production will ultimately be expressed in many small fish or a moderate increase in the number of larger fast growing fish. From a fisheries perspective, the first alternative is certainly not desirable.

Addition of phosphorus to one of the two basins of a lake referred to as Lake 226 (Experimental Lakes Area) rapidly increased lake whitefish (*Coregonus clupeaformis*) growth, survival, biomass and production, but 3 yr after fertilization, only biomass differences remained, growth, survival, and production having returned to pretreatment levels (Mills and Chalanchuk 1987). There have also been occasions when fertilization did not increase fish production or growth (Smith 1961). Nakashima and Leggett (1975) found that perch growth did not differ significantly along a pronounced natural trophic-phosphorus gradient in Lake Memphremagog, even though fish biomass varied by two-fold in response to changes in biomass and production at lower trophic levels.

While the results of our study do not explain these differences, they do suggest that the assumption that increasing only the quantity of food available will lead to long-term increases in growth rates may be flawed.

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Among-Population Variability of Fish Growth: II. Influence of Prey Type.[1]

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Boisclair, D., and W. C. Leggett. 1989. Among-population variability of fish growth: II. Influence of prey type. Can. J. Fish. Aquat. Sci. 46: 468–482.

We tested the significance of the relationship between in situ growth and several diet descriptors using 12 yellow perch (*Perca flavescens*) populations that varied widely in growth rates. In addition, we tested the assumption of optimal foraging theory that mean prey size is an important regulator of fish growth. The percent contribution of four prey taxonomic groups and six prey size–classes (alone or combined with food consumption estimates) explained between 41 and 95% of among-population variability in growth rates or growth efficiency. We found no significant relationship between growth rates or growth efficiency and mean prey weight. We observed a pattern suggesting the existence of an optimal prey size for (1 mg dry weight) age I+ and age II+ fish, when the percent contribution of specific prey taxonomic groups or size–classes to the diet was assessed. This pattern was not apparent in the relationship between growth and the mean weight of prey consumed. The energetic advantage of feeding on a given prey type is believed to be related to its low cost/benefit ratio while foraging. Our data indicate that interactions between prey availability (index of searching component of foraging cost) and prey size (index of handling component of foraging cost) are more effectively represented by the percent contribution of a given prey type to the diet than by the mean weight of prey consumed. Our analyses lead us to conclude that the probability of deriving broadly applicable models explaining among-population variability in fish growth rates based on mean prey weight is very low.

Nous avons examiné l'intensité de la relation entre la croissance *in situ* et plusieurs descripteurs de la diète en utilisant 12 populations de perchaudes (*Perca flavescens*) dont la croissance varie grandement. Nous avons aussi tenté de vérifier une prémisse de la théorie de la quête optimale de nourriture selon laquelle la taille moyenne des proies est un régulateur important de la croissance des poissons. Le pourcentage de la contribution de quatre groupes taxonomiques de proies et de six classes de tailles de proies (seuls ou combinés à des estimés de consommation de nourriture) ont expliqué entre 41, et 95% de la variabilité inter-population du taux de croissance et de l'efficacité de croissance. Nous n'avons pas trouvé de relation significative entre la croissance ou l'efficacité de croissance et la taille moyenne des proies ingérées. Nous avons observé un patron suggérant l'existence d'une taille optimale de proies pour les poissons d'âge I+ et II+ (1 mg poids sec) lors de l'analyse de l'influence de la contribution de certains groupes taxonomiques ou classes de tailles de proies sur la croissance. Ce patron n'a pas été apparent lorsque les relations entre la croissance et la taille moyenne des proies ont été examinées. L'avantage énergétique de consommer un type donné de proie semble résider dans un faible rapport coût/bénifice lors de la nutrition. Nos données indiquent que les interactions entre la disponibilité des proies (indice du coût de recherche) et leur taille (indice du coût de manipulation) sont représentées plus adéquatement par la contribution d'un type donné de proies à la diète que par la taille moyenne des proies ingérées. Nos analyses mènent à la conclusion que la probabilité de trouver un modèle général pour expliquer les variations inter-populations de la croissance des poissons à l'aide de la taille moyenne des proies est très faible.

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We recently reported the results of studies which show that the quantity of food consumed by fish explains only a small proportion (52% for age I+, 0% for age II+, and 0% for age III+) of among-population variability in fish growth in situ (Boisclair and Leggett 1989). A popular alternate hypothesis regarding the causes of among-population variability in fish growth concerns the character of the prey comprising their respective diets. Two lines of enquiry support this hypothesis.

Direct Proportionality Models

Several empirical studies, both laboratory and field, have demonstrated that fish, when presented with choice, tend to select the largest prey items available (Hrbacek 1962; Brooks and Dodson 1965; Reif and Tappa 1966; Galbraith 1967; Brooks 1968; Wells 1970; Hutchinson 1971; and see Lazarro 1987 for a review). Furthermore, it is expected that those fish that most consistently select the largest food items should grow fastest

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(Elton 1927; Ivlev 1961; MacLean and Magnuson 1977). Many laboratory (Paloheimo and Dickie 1966; Wankowski and Thorpe 1979; Hart and Connellan 1984) and field experiments (Alm 1946; Martin 1966; Mills and Forney 1981; Mousseau 1983; Rask 1983; Rask and Hiisivuori 1985) have provided data consistent with the hypothesis of a positive relationship between fish growth and prey size. The effect of prey type on growth has also been inferred from the frequently observed negative effect of fish density on fish growth. This result is believed to occur because high fish densities depress prey quality (Alm 1946, 1952; LeCren 1958; Nilsson 1972; MacLean and Magnuson 1977; Hansson 1985).

Optimality Models

A series of models based on optimal foraging theory, and ultimately constructed to predict fish diet in situ, have refined the interpretation of the observed relationship between growth and prey size (MacArthur and Pianka 1966; Emlen 1966; Schoener 1969, 1971; Pulliam 1974; Werner and Hall 1974; Charnov 1976; Estabrook and Dunham 1976; Eggers 1977; Norberg 1977; Hugues 1979; Anholt et al. 1987). These models, while based on slightly different assumptions, especially those regarding the currency to be optimized (handling time/prey weight; energy of capture/prey caloric content; net energy gain/energy of capture; net energy gain/unit of time; Stein et al. 1984; Stevens and Krebs 1986) and the mechanics of prey perception (O'Brien et al. 1976, 1985; Gibson 1980; Butler and Bence 1984; Wetterer and Bishop 1985), are inherently similar in that they are all based on the idea that prey profitability reaches an optimum level at intermediate prey sizes. While successfully applied in many cases (Werner and Hall 1974; Kislalioglu and Gibson 1976a; Stein 1977; Werner 1979; Ringler 1979; Gillen et al. 1981; Mittelbach 1983) prey selection models based on optimal foraging theory frequently fail to predict fish diet (Hall et al. 1979; Furnass 1979; Hansen and Wahl 1981; Magnhagen and Wiederholm 1982; Stein et al. 1984).

Despite differences in their predictions, the direct proportionality and optimality models are compatible since the former can be considered as special cases of the latter when a smaller range of prey size is examined. Most importantly, both types of models suggest the existence of a strong relationship between fish growth and prey size.

In this study, we attempted to directly verify whether among-population variability in in situ fish growth rates and growth efficiency could be adequately explained by prey type descriptors. Using previously published data (Boisclair and Leggett 1989), we further examined the combined effect of prey type and quantity of food consumed on fish growth.

Methods

Species and Populations Studied

The species chosen for study was the yellow perch (*Perca flavescens*). Preliminary sampling of lakes of the Eastern Townships region of Quebec during 1984 identified 12 populations (Fig. 1) exhibiting a range of growth rates that approached the full range reported for perch at this latitude (Grimaldi and Leduc 1973; Nakashima and Leggett 1975; Heath and Roff 1987; Boisclair and Leggett 1989). The morphological and limnological characteristics of the lakes surveyed are described in Rasmussen (1988 a,b) and Boisclair and Leggett (1989).

Sampling

Sampling of fish diets was executed coincident with the collection of samples used to estimate food consumption and growth (Boisclair and Leggett 1989). Data were sufficient to permit analysis of the growth rates of I+ fish from eight lakes,

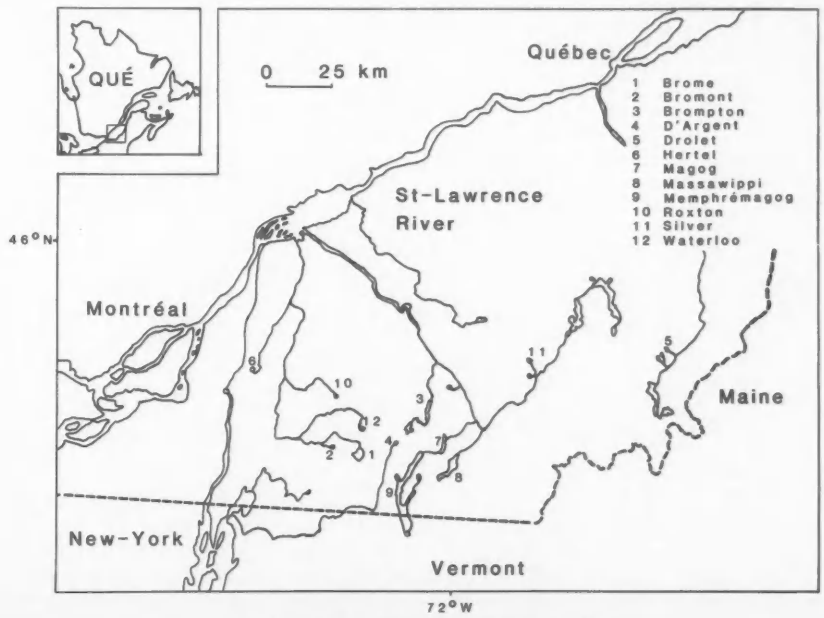

FIG. 1. Study area and the populations sampled.

TABLE 1. Relative growth rate (RGR; g wet weight · g wet weight^{-1}· d^{-1}) and growth efficiency (K_1; per cent) for each combination of perch age–class and population studied ($A \cdot P$; Boisclair and Leggett 1989).

$A \cdot P$	RGR	K_1
Memphremagog I	0.014	24.5
Silver I	0.014	23.0
Drolet I	0.013	21.2
Massawippi I	0.013	13.9
Brompton I	0.010	16.1
Waterloo I	0.010	47.3
D'Argent I	0.009	25.3
Bromont I	0.008	27.6
Silver II	0.010	16.1
Magog II	0.009	12.9
Memphremagog II	0.008	12.9
Brompton II	0.007	10.4
Roxton II	0.006	10.2
Waterloo II	0.004	16.8
Hertel II	0.003	4.2
Bromont II	0.003	9.7
Massawippi II	0.002	2.3
D'Argent II	0.001	3.5
Waterloo III	0.006	29.2
Memphremagog III	0.005	8.2
Drolet III	0.004	6.5
Roxton III	0.004	6.7
Magog III	0.004	4.9
Brompton III	0.003	4.5
Brome III	0.002	2.2
D'Argent III	0.002	5.6
Hertel III	0.002	2.8
Bromont III	0.002	5.5

TABLE 2. Prey taxonomic groups used to describe perch diet. Values in parentheses represent the percentage of a given taxonomic group falling within a given size–class (refer to Table 5 for the definition of prey size–classes).

Taxonomic groups	Abbrev.	Contribution by size–classes (% by weight)
Amphipoda	amp	SC7 (58.3) SC6 (16.7) SC5 (15.4)
Coleoptera	col	SC7 (53.3) SC8 (42.2)
Decapoda	dec	SC9 (96.4)
Diptera	dip	SC5 (38.5) SC7 (35.0) SC6 (20.5)
Ephemeroptera	eph	SC8 (18.5) SC7 (18.1) SC10(18.1)
Gastropoda	gas	SC7 (46.0) SC5 (26.1) SC6 (21.3)
Hemiptera	hem	SC6 (39.3) SC7 (29.8) SC5 (29.5)
Hydracarina	hyd	SC5 (100.0)
Odonata	odo	SC11(35.8) SC9 (33.2) SC8 (16.2)
Oligochaeta	oli	SC5 (100.0)
Ova (fish eggs)	ova	SC5 (89.5)
Pelecypoda	pel	SC9 (44.2) SC6 (23.0) SC7 (20.1)
Pisces	pis	SC11(88.1)
Rana	ran	SC11(100.0)
Tricoptera	tri	SC8 (25.8) SC10(23.5) SC7 (15.9)
Zooplankton	zo	SC3 (60.0) SC2 (27.4)

and II+ and III+ fish from 10 populations (Boisclair and Leggett 1989). Growth rates in the populations sampled varied by 1.8-(age I+ fish) to 10-fold (age II+ fish; Table 1).

Description of the Diet

Between 15 and 20 perch, ranging from 50 to 150 mm total length (TL), and having full stomachs, were retained from each population on each sampling date. Fish with full stomachs were selected to ensure that food items would be intact and easy to identify. Total length (millimetres) of each fish was determined and its diet was documented by photographing the stomach contents in the field within 3 h of capture. For photography, the contents of each stomach were spread on 3 cm × 5 cm plexiglass trays (0.4-cm thick) which were then filled with water. A black tray bottom was used to maximize contrast. A 35-mm Contarex camera fitted with a 2× adapter was mounted such that the field of view corresponded exactly with the limits of the tray. Fish length was recorded in each photograph and a 1-cm ruler graduated to 1 mm was photographed as a reference at the beginning of each series of photographs.

For analysis, images of the gut contents of individual fish were projected on a digitizer (Houston Instrument) calibrated with reference to the ruler scale on each picture series. All prey items were identified and measured to the nearest 0.1 mm. Prey length was transformed to dry weight using allometric relationships derived empirically for the lakes studied (J. B. Rasmussen, McGill University, unpubl. data). For Odonata and Tricoptera the relationships given by Smock (1980) were used. In this paper prey size is thus synonymous with prey weight. Prey were classified into 16 taxonomic groups and 11 size– (weight) classes (Table 2 and 5).

The percent contribution of each prey type to the diet on a given sampling day i (p_{ij} ; percent) was estimated as

$$(1)\quad p_{ij} = \frac{\sum_{n=1}^{N} w_{nj}}{\sum_{j=1}^{J}\sum_{n=1}^{N} w_{nj}} \times 100$$

where w_{nj} is the weight of an individual prey of taxonomic group or weight class j, J is the total number of taxonomic groups (16) or weight–classes (11) collected, and N is the total number of prey of that type. The absolute quantity of a given prey type consumed by perch of each age–class in each population during a sampling day (W_{ij}; g dry weight·d^{-1}) was estimated as

$$(2)\quad W_{ij} = \frac{p_{ij}}{100} \times D_i$$

where D_i is the absolute quantity consumed per day by perch of given combination of age–class and population (g dry weight·d^{-1}). The values D_i as reported in Boisclair and Leggett (1989) were used in the analysis. The seasonal percent contribution of a given prey type to the total biomass of food consumed by a given combination of perch age–class and population (P_j; percent) was estimated as

$$(3)\quad P_d = \frac{\sum_{i=1}^{I} W_{ij}}{\sum_{i=1}^{I}\sum_{j=1}^{J} W_{ij}} \times 100$$

where I is the number of sampling sessions executed for a given population (i.e. four).

Mean prey weight on any given sampling day (w_{mi}) was estimated for each combination of perch age–class and population as the arithmetic mean weight of all prey consumed by that combination of perch age–class and population during day i.

Mean prey weight (MPW: milligrams dry weight) was estimated as

$$(4)\quad \mathrm{MPW} = \sum_{i=1}^{I} \frac{D_i}{\sum_{i=1}^{I} D_i} \times W_{mi}$$

A predator to prey weight ratio (Q) was estimated for each combination of perch age–class and population as

$$(5)\quad Q = \frac{\mathrm{MPW}/1000}{W_m}$$

where W_m is the mean (dry) weight of perch of a given combination of age–class and population during the interval between the first and last sampling session (Boisclair and Leggett 1989). We used a wet to dry weight coefficient of 5.07 ($s = 1.10$; $n = 20$) to convert fish wet weight to dry weights.

Diet diversity (expressed both as taxonomic groups and size–classes) was estimated for each combination of perch age–class and population using the Shannon-Weaver diversity index (H Legendre and Legendre 1979).

$$(6)\quad H = -\sum_{j=1}^{J} P_j \cdot \log_{10} P_j$$

This index has a minimum value of 0 (for diets composed of a single prey) and a maximum value of 1 (for diets composed of several prey all of which have equal importance).

Relationship between Growth Rates and Diet Descriptors

Relationships between growth rates and the percent contribution of a given prey type to the diet of perch were determined for each age–class independently using regression analysis. We similarily examined the relationship between growth rates and (1) mean prey weight; and (2) diet diversity indices. To qualify as an independent variable, we required that a given prey taxonomic group or size–class occur in the diet of >50% of the populations sampled, as to comprize >10% of the diet of perch. The quantity of food consumed (Boisclair and Leggett 1989) was also used as an independent variable in some analyses. In these cases we used multiple and stepwise multiple regression techniques. Relative growth and relative consumption rates were used in these analyses. This was done for consistency with previous studies, and to preclude spurious relationships resulting exclusively from the covariation of growth and food consumption rates with fish size (Boisclair and Leggett 1989). Analyses were performed using untransformed data, as well as data converted to natural logarithms and squared values (to account for potential curvilinearity in the relationships). We retained only those models in which all variables identified contributed significantly ($p < 0.05$) to the regression.

Relationship between Growth Efficiency and Diet Descriptors

The relationships between growth efficiency and diet descriptors were examined using the same analytical procedure employed for growth rates. In these analyses absolute rather than relative consumption rates were used (Paloheimo and Dickie 1966; Boisclair and Leggett 1989).

As noted in Boisclair and Leggett (1989), growth efficiency estimates for Lake Waterloo were suspiciously high (Table 1). Our analyses of the contribution of diet descriptors to the explained variance in growth efficiency were therefore performed both with and without data from that lake. Although the exclusion of Lake Waterloo did alter the coefficients of the models, it did not influence the form of the relationships observed, or the conclusions drawn.

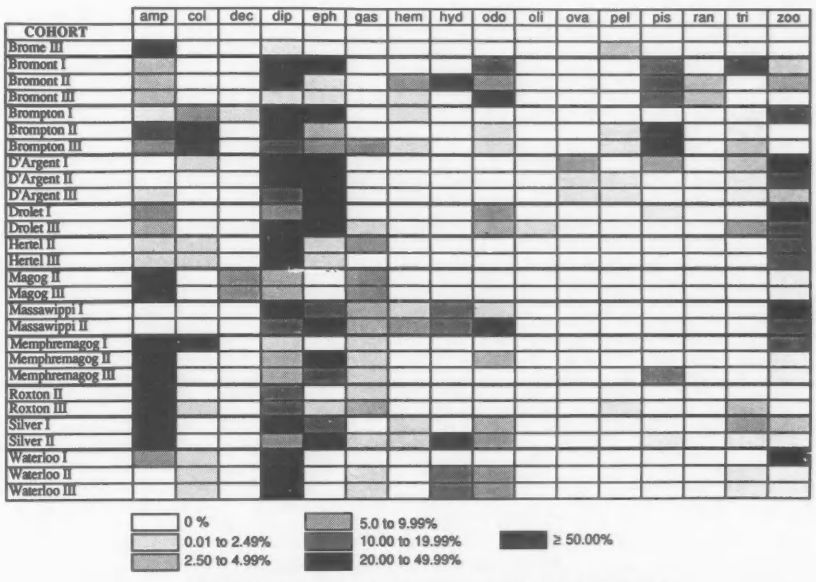

FIG. 2. Importance of prey taxonomic groups to the diet of each combination of perch age–class and population studied (refer to Table 2 for the abbreviations of taxonomic groups).

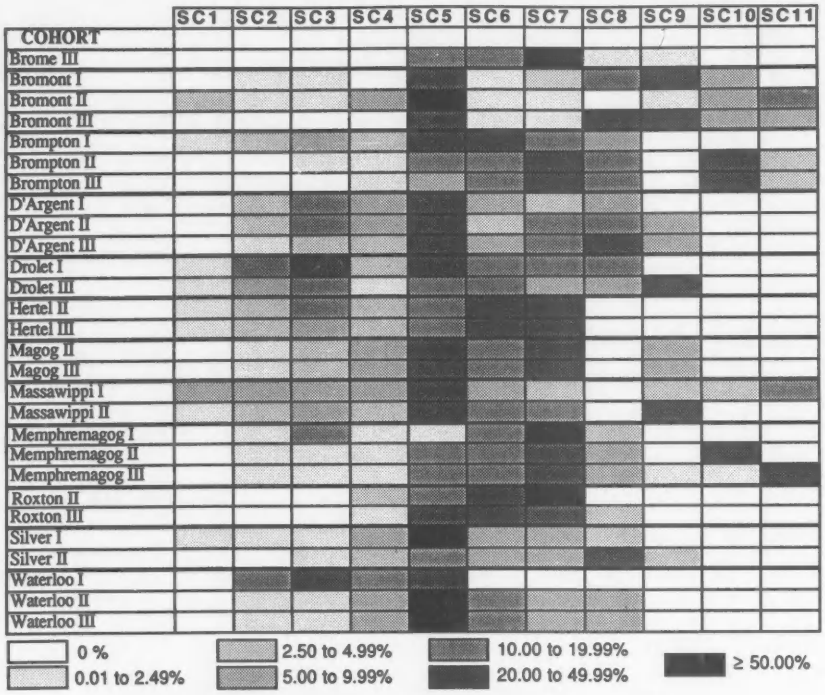

FIG. 3. Importance of prey size–classes to the diet of each combination of perch age–class and population studied (refer to Table 5 for the definition of size–classes).

TABLE 3. Contribution of taxonomic groups to perch diet. Occurrence is the proportion of combinations of perch age–class and population ($A \cdot P$) containing a given taxonomic group in the diet.

Taxonomic groups	Occurrence (%)	Maximum contribution (% weight in diet ($A \cdot P$))	Mean contribution (% weight in diet)
Dip	100.0	78.76 (Waterloo III)	26.59
Amp	78.6	95.20 (Brome III)	25.62
Eph	71.4	75.92 (D'Argent III)	13.91
Zoo	78.6	59.32 (Waterloo I)	10.31
Odo	50.0	46.26 (Bromont III)	4.76
Hyd	25.0	34.56 (Bromont II)	4.59
Pis	25.0	27.58 (Brompton II)	4.22
Col	39.3	37.86 (Memphremagog I)	4.22
Gas	57.1	9.23 (Magog II)	1.91
Tri	46.4	17.24 (Bromont I)	1.74
Hem	28.6	7.10 (Massawippi II)	0.64
Dec	10.7	7.89 (Magog II)	0.56
Pel	28.6	3.03 (Brome III)	0.35
Ran	7.1	4.47 (Bromont II)	0.30
Ova	10.7	5.37 (D'Argent I)	0.26
Oli	3.6	0.16 (Drolet III)	0.01

Results

Description of the Diet

A total of 23 787 prey were identified and measured. The relative importance of each prey taxonomic group and size-class to the diet was described for age I+ fish from eight populations, II+ fish from 10 populations, and III+ fish from 10 populations (Fig. 2, Fig. 3). The diets of the different populations and age–classes varied dramatically in terms of both taxonomic groups and size–classes.

Prey Taxonomic Groups

Fourteen of the 16 prey taxonomic groups identified had more than 80% of their biomass distributed in three size–classes (Table 2). Hydracarina, Oligochaeta, and Rana were each completely subsumed by a single size–class (size–classes 5, 5, and 11, respectively).

TABLE 4. Contribution of prey taxonomic groups to the diet of each combination of perch age–class and population ($A \cdot P$) studied. Richness is the percentage of prey taxonomic groups that contributed to the diet. Maximum contribution is the maximum contribution of a single prey type to the diet. Taxonomic diversity indices of the diets are also presented.

		Maximum contribution		
$A \cdot P$	Richness (%)	% Weight in diet	Taxonomic groups	Diversity (0–1)
Memphremagog I	31.25	44.03	(Amp)	0.49
Silver I	43.75	41.22	(Amp)	0.62
Drolet I	37.50	52.74	(Zoo)	0.57
Massawippi I	43.75	42.47	(Dip)	0.64
Brompton I	50.00	45.30	(Dip)	0.57
Waterloo I	25.00	59.32	(Zoo)	0.39
D'Argent I	43.75	38.26	(Dip)	0.67
Bromont I	43.75	32.76	(Dip)	0.74
Silver II	62.50	33.88	(Amp)	0.71
Magog II	31.25	79.53	(Amp)	0.32
Memphremagog II	37.50	65.64	(Amp)	0.45
Brompton II	50.00	31.81	(Col)	0.67
Roxton II	18.75	85.24	(Amp)	0.21
Waterloo II	43.75	78.34	(Dip)	0.36
Hertel II	37.50	75.00	(Dip)	0.38
Bromont II	56.25	34.50	(Hyd)	0.76
Massawippi II	43.75	25.98	(Odo)	0.77
D'Argent II	37.50	44.58	(Eph)	0.54
Waterloo III	43.75	78.76	(Dip)	0.35
Memphremagog III	37.50	67.74	(Amp)	0.44
Drolet III	50.00	44.21	(Eph)	0.63
Roxton III	43.75	72.37	(Amp)	0.41
Magog III	31.25	80.84	(Amp)	0.31
Brompton III	62.50	33.53	(Col)	0.77
Brome III	18.75	95.20	(Amp)	0.10
D'Argent III	43.75	75.92	(Eph)	0.37
Hertel III	37.50	75.76	(Dip)	0.37
Bromont III	50.00	46.26	(Odo)	0.60

The proportion of the fish that had consumed prey of a specific taxonomic group ranged from 3.6 for Oligochaeta to 100.00% for Diptera (Table 3). The maximum contribution by a single taxonomic group to the diet of a given combination of perch age–class and population ranged from 0.16 (Oligochaeta, age III+, Lake Drolet) to 95.20% (Amphipoda, age III+, Lake Brome). The mean contribution of a given taxonomic group to all combinations of perch age–class and taxonomic group ranged from 0.01 (Oligochaeta) to 26.59% (Diptera).

The proportion of the taxonomic groups identified (Table 4) that contributed to the diet of a given combination of perch age–class and population ranged from 18.75 (age II+, Lake Roxton; age III+, Lake Brome) to 62.50% (age II+, Lake Silver; age III+, Lake Brompton). The maximum contribution of a single taxonomic group to the diet of a given combination of perch age–class and population ranged from 25.14 (Odonata, age II+, Lake Massawippi) to 95.20% (Amphipoda, age III+, Lake Brome). There was no significant relationship between the percent contribution of any prey taxonomic group and mean fish weight within age–classes ($p < 0.05$).

Taxonomic diversity ranged from 0.10 (age III+, Lake Brome) to 0.77 (age II+, Lake Massawippi; age III+, Lake Brompton), and averaged 0.59 for I+, 0.52 for II+, and 0.43 for III+ fish (Table 4). Taxonomic diversity varied significantly among populations ($F_{11,14} = 7.18$; $p < 0.0005$) but not among age–classes ($F_{2,14} = 0.08$; $p > 0.90$). We found no significant relationship between taxonomic diversity and mean fish weight. This was true both within age–classes (Table 8) and when age–classes were pooled ($F_{1,26} = 0.22$; $p > 0.60$).

Prey Size Classes

The prey size–classes we identified were strongly associated with a small number of taxonomic groups (Table 5). Size–classes 1–3 were exclusively zooplankton. Size–classes 4–7 were comprized of Amphipoda and Diptera, and size–classes 8–11 contained Odonata, Ephemeroptera, and Pisces.

The proportion of fish exhibiting a given prey size–class in their diets ranged from 32.1 for size class 1 to 100.00% for size–class 5 (Table 6). The maximum contribution by a single size–class to the diet of a given combination of perch age–class and population ranged from 6.37 (size–class 1, age I+, Lake Massawippi) to 68.89% (size–class 5, age III+, Lake Waterloo). The mean contribution of a given size–class to all fish sampled ranged from 0.58 (size–class 1) to 29.88% (size–class 5).

The proportion of the total complement of prey size–classes (Table 7) that contributed to the diet of a single combination of perch age–class and population ranged from 36.36 (age I+, Lake Waterloo; age II+, Lake Roxton) to 90.91% (age I+, Lake Massawippi; age II+, Lake Bromont; age III+, Lake Memphremagog). The maximum contribution by any prey size–

TABLE 5. Prey size–classes used to describe perch diet. Values in parentheses represents the proportion of a size–class failing within a given taxonomic groups (refer to Table 2 for the abbreviations of taxonomic groups).

Size–classes	Limits (mg dry weight)	Abbrev.	Contribution by taxonomic groups (% by weight)			
1	0.0000–0.0049	SC1	Zoo	(99.7)		
2	0.0050–0.0149	SC2	Zoo	(98.4)		
3	0.0150–0.0499	SC3	Zoo	(82.8)		
4	0.0500–0.0999	SC4	Dip	(45.4)	Amp	(34.2)
5	0.1000–0.499	SC5	Dip	(44.3)	Amp	(33.3)
6	0.5000–0.9999	SC6	Amp	(49.7)	Dip	(32.5)
7	1.0000–4.9999	SC7	Amp	(60.7)	Dip	(19.4)
8	5.0000–14.9999	SC8	Odo	(24.7)	Eph	(23.5)
9	15.0000–29.9999	SC9	Odo	(58.1)	Eph	(23.3)
10	30.0000–49.9999	SC10	Pis	(45.6)	Eph	(25.7)
11	>50.0000	SC11	Pis	(81.0)		

TABLE 6. Contribution of size–classes to perch diet. Occurrence is the percentage of combinations of perch age–class and population ($A \cdot P$) in which a given size–class occurred in the diet.

	Occurrence (%)	Maximum contribution (% weight in diet ($A \cdot P$))	Mean contribution (% weight in diet)
SC5	100.0	68.89 (Waterloo III)	29.88
SC7	96.4	67.31 (Brome III)	23.95
SC6	96.4	35.00 (Brompton I)	12.68
SC8	67.9	43.74 (Silver II)	8.06
SC3	82.1	40.68 (Waterloo I)	6.73
SC9	46.4	35.04 (Bromont I)	5.65
SC4	89.3	13.56 (Waterloo I)	4.17
SC10	28.6	24.25 (Brompton II)	3.57
SC2	75.0	18.64 (Waterloo I)	2.62
SC11	21.4	21.72 (Memphremagog III)	2.13
SC1	32.1	6.37 (Massawippi I)	0.58

TABLE 7. Contribution of prey size–classes to the diet of each combination of perch age–class and population ($A \cdot P$) studied either in terms of richness (percentage of prey size–classes that contributed to the perch diet), and maximum contribution (size–classes concerned appears in parentheses). Mean prey weight (MPW) and size–class diversity indices are also presented.

		Maximum contribution			
$A \cdot P$	Richness (%)	% Weight in diet	Size–classes	MPW (mg dry weight)	Diversity (0–1)
Memphremagog I	72.73	65.56	(SC7)	1.13	0.50
Silver I	72.73	68.12	(SC5)	0.15	0.51
Drolet I	72.73	22.13	(SC5)	0.15	0.81
Massawippi I	90.91	39.81	(SC5)	0.22	0.84
Brompton I	72.73	35.00	(SC6)	0.13	0.71
Waterloo I	36.36	40.58	(SC3)	0.03	0.57
D'Argent I	63.64	49.65	(SC5)	0.12	0.66
Bromont I	72.73	35.04	(SC9)	0.88	0.66
Silver II	63.64	43.74	(SC8)	0.60	0.59
Magog II	63.64	40.47	(SC7)	0.21	0.65
Memphremagog II	72.73	40.04	(SC7)	0.54	0.68
Brompton II	72.73	32.48	(SC7)	1.87	0.69
Roxton II	36.36	43.18	(SC7)	0.55	0.47
Waterloo II	63.64	68.39	(SC5)	0.20	0.49
Hertel II	63.64	42.71	(SC7)	0.75	0.66
Bromont II	90.91	63.99	(SC5)	1.11	0.56
Massawippi II	72.73	32.06	(SC5)	0.10	0.75
D'Argent II	72.73	35.09	(SC5)	0.66	0.79
Waterloo III	63.64	68.89	(SC5)	0.22	0.48
Memphremagog III	90.91	39.08	(SC7)	2.79	0.71
Drolet III	81.82	33.26	(SC9)	0.21	0.80
Roxton III	45.45	43.18	(SC7)	0.51	0.54
Magog III	63.64	40.19	(SC7)	0.37	0.65
Brompton III	63.64	39.30	(SC7)	0.85	0.67
Brome III	45.45	67.31	(SC7)	1.11	0.42
D'Argent III	72.73	38.00	(SC5)	2.06	0.73
Hertel III	63.64	43.20	(SC7)	0.35	0.66
Bromont III	63.64	33.14	(SC5)	3.55	0.70

class to the diet of a single combination of perch age–class and population ranged from 22.13 (size–class 5, age I+, Lake Drolet) to 68.89% (size class 5, age III+ Lake Waterloo). The contribution to fish diet was significantly related to mean fish weight in only a few size–classes; the percent contribution of size–class 1 to fish diet decreased significantly with mean weight of age III+ fish ($F_{1,8} = 6.74$; $p < 0.05$), while that of size–class eight increased significantly with mean weight of age II+ fish ($F_{1,8} = 22.41$; $p < 0.002$).

Mean prey weight (MPW) ranged from 0.03 mg dry weight (age I+, Lake Waterloo) to 3.55 mg dry weight (age III+, Lake Bromont). MPW varied among populations by 38-fold for age I+ fish, 19-fold for age II+ fish, and by 16-fold for age III+ fish (Table 7). These differences were statistically significant both among populations and among age–classes (Table 9). We found no significant relationship between MPW and mean fish weight within age–classes (Table 8) or when age–classes were pooled ($F_{1,26} = 3.55$; $p > 0.05$).

Prey to predator weight ratios ranged from 2.0×10^{-5} (age I+, Lake Waterloo) to 7.2×10^{-4} (age I+, Lake Bromont) and varied among populations by 34-fold for age I+ fish, 12-fold for age II+ fish, and 12-fold for age III+ fish. Prey to predator weight ratios did not vary significantly among populations ($F_{11,14} = 2.07$; $p > 0.10$) or age–classes ($F_{2,14} = 0.06$;

TABLE 8. Statistics of the relationships between taxonomic diversity, mean prey weight, or size–class diversity and mean fish weight. Degrees of freedom associated with F values are 1,6 for age I+ fish, and 1,8 for age II+ and III+ fish.

	F	p
Taxonomic diversity		
I+	0.05	>0.80
II+	0.86	>0.30
III+	0.81	>0.40
Mean prey weight		
I+	0.27	>0.60
II+	0.01	>0.99
III+	0.13	>0.70
Size–class diversity		
I+	0.16	>0.70
II+	0.06	>0.80
III+	1.20	>0.30

TABLE 9. (a) Statistics of the among-population and among- age–class variability in mean prey weight (two-way ANOVA). (b) Significance of among-population variability in mean prey weight for each age–class (one-way ANOVA).

	F	df	p
(a) Factor			
Population	16.95	11,23759	<0.0001
Age–class	27.44	2,23759	<0.0001
Interaction	5.92	14,23759	<0.0001
(b) Age I+	3.92	7,12654	<0.0005
Age II+	15.68	9,5771	<0.0001
Age III+	29.46	9,5343	<0.0001

$p > 0.90$). This led us to conclude that among-population variability in the prey to predator ratio could not be responsible for variability in growth rates or growth efficiency.

The size–class diversity of the diet ranged from 0.42 (age III+, Lake Brome) to 0.84 (age I+, Lake Massawippi) and averaged 0.66 for age I+ fish, and 0.64 for age II+ and age III+ fish (Table 7). Size–class diversity varied significantly among populations ($F_{11,14} = 6.19$; $p < 0.002$) but not among age–classes ($F_{2,14} = 0.36$; $p > 0.70$). We found no significant relationship between size–class diversity and mean fish weight within age–classes (Table 8) or when age–classes were pooled ($F_{1,26} = 0.03$; $p > 0.80$).

Relationship between Growth Rates and Prey Taxonomic Groups

Only three of the 16 taxonomic groups examined explained a significant proportion of the observed among-population variability in fish growth rates. In these cases the variance explained ranged from 48 to 87% (Table 10a). Growth rates of I+ ($F_{1,6} = 7.61$) and II+ fish ($F_{1,8} = 7.03$) increased as the contribution of Amphipoda in the diet increased. A significant quadratic relationship existed between the growth rates of age II+ fish and the contribution of Amphipoda ($F_{2,7} = 23.80$) and Diptera to the diet ($F_{2,7} = 8.19$). The shape of these relationships (within the observed range of prey contribution to the diet) indicated that growth rates of age II+ fish increased as the contribution of Amphipoda increased but decreased as the contribution of Diptera increased. Growth rates of age II+ fish decreased exponentially as the percent contribution of zooplankton to the diet increased ($F_{1,8} = 25.69$). An analysis of the effect of prey size within these taxonomic groups (Amphipoda, Diptera, and zooplankton) was performed. No significant influence was revealed.

Inclusion of the quantity of food consumed as a variable in models linking the percent contribution of prey taxonomic groups to the growth rates of age I+ and II+ perch increased the proportion of the variance explained by 11–31% (Table 10b). Addition of the quantity of food consumed to the list of independent variables revealed two new relationships for age I+ fish (Table 10b). Perch growth rates always increased as ration increased.

Relationship between Growth Rates and Taxonomic Diversity of the Diet

There was no significant relationship between growth rates and taxonomic diversity of the diet (age I+: $F_{1,6} = 0.63$; p

TABLE 10. Significant relationships ($p < 0.05$) observed between (a) growth rates (RGR) and diet descriptors (refer to Tables 2 and 5 for the definition of prey taxonomic groups and size–classes) and (b) between growth rates, diet descriptors, and food consumption estimates (RDR; Boisclair and Leggett 1989).

Age–classes	Models	r^2	p
(a) I+	$RGR = 0.0001\ AMP + 0.01$	0.56	0.03
II+	$\ln RGR = 0.013\ AMP - 5.82$	0.48	0.03
	$RGR = -0.00009003\ AMP^2 + 0.0003\ AMP + 0.002$	0.87	0.0008
	$RGR = 0.000004\ DIP^2 - 0.0004\ DIP + 0.01$	0.70	0.01
	$\ln RGR = -0.089\ ZOO - 4.97$	0.76	0.001
	$\ln RGR = -0.31\ SC2 - 5.06$	0.49	0.02
	$\ln RGR = -0.11\ SC3 - 5.00$	0.61	0.007
III+	none		
(b) I+	$RGR = 0.0001\ AMP + 0.43\ RDR + 0.0007$	0.87	0.006
	$RGR = -0.000002\ DIP^2 + 0.56\ RDR + 0.009$	0.79	0.02
	$RGR = -0.00013\ EPH + 0.004 \ln RDR + 0.34$	0.83	0.01
	$RGR = 0.000003\ SC5^2 - 0.00025\ SC5 + 0.44\ RDR + 0.01$	0.87	0.03
II+	$\ln RGR = -0.09\ ZOO + 0.50 \ln RDR - 2.51$	0.87	0.0007
	$\ln RGR = -0.38\ SC2 + 0.87 \ln RDR - 0.71$	0.80	0.004
III+	none		

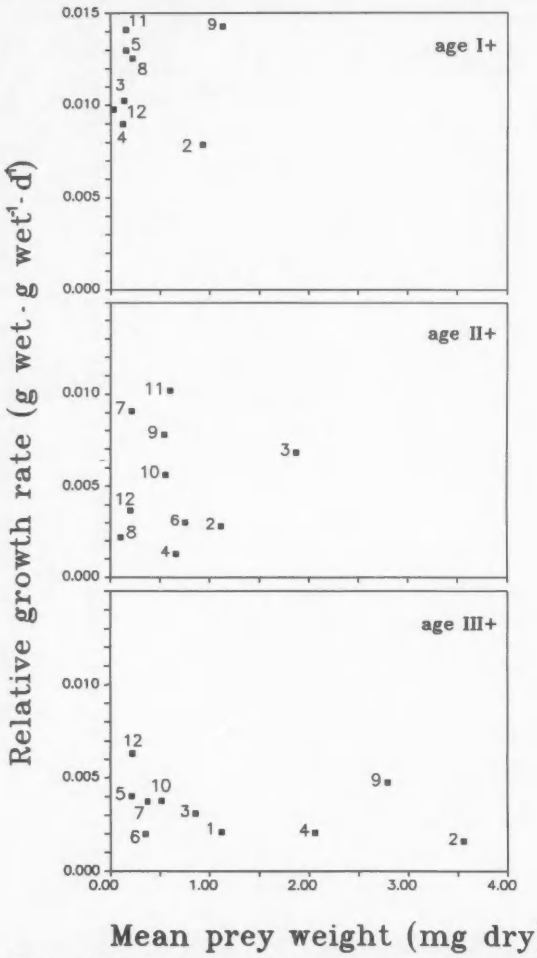

FIG. 4. Relationship between relative growth rates and mean prey weight for each age–class studied. The populations are identified using the legend in Fig. 1.

>0.40; age II+: $F_{1,8}$ =0.14; p >0.70; age III+: $F_{1,8}$ =0.002; p >0.95). The inclusion of food consumption estimates in the models did not alter this conclusion.

Relationship between Growth Rates and Prey Size–Classes

Of the 11 prey size–classes examined, size–classes 2, 3, and 8 explained a significant proportion of among-population variability in growth rates of age II+ and III+ fish (Table 10a). Growth rates of age II+ fish decreased exponentially as the percent contribution of size–classes 2 ($F_{1,8}$ =7.78) and 3 ($F_{1,8}$ =12.75) increased in the diet.

The addition of quantity of food consumed as a variable in models relating growth rates to the percent contribution of prey size–classes explained an additional 31% of the observed among-population variability of age II+ fish (Table 10b). The only new relationship revealed by the inclusion of feeding level to the list of independent variables explored was that for age I+ fish and involved size–class 5 (Table 10b). Perch growth rates always increased as feeding level increased.

Relationship between Growth Rates and Mean Prey Weight

We found no significant relationship between fish growth rates and mean prey weight (age I+: $F_{1,6}$ =0.05; p >0.80; age II+: $F_{1,8}$ =0.02; p >0.90; age III+: $F_{1,8}$ =1.28; p >0.20). The inclusion of food consumption estimates in the models did not influence these results. There was no evidence of the existence of an optimal prey size (Fig. 4).

Relationship between Growth Rates and the Diversity of Size Classes in the Diet

We found no significant relationship between fish growth rates and size–class diversity of the diet (age I+: $F_{1,6}$ =0.14; p >0.70; age II+: $F_{1,8}$ =0.27; p >0.60; age III+: $F_{1,8}$ =0.28; p >0.60). The addition of food consumption estimates to diet size–class diversity in the models revealed no additional significant relationships.

Relationship between Growth Efficiency and Prey Taxonomic Groups

Of the 16 taxonomic groups examined only the percent contribution of Amphipoda and zooplankton to the diet explained a significant proportion of among-population variability of growth efficiency (Table 11a). We found a significant quadratic relationship between the growth efficiency of both age II+ and age III+ fish and the percent contribution of Amphipoda to their diets (age II+: $F_{2,6}$ =16.34; age III+: $F_{2,6}$ =5.54). The shape of these models, in the observed range of prey contribution to the diet, suggests that growth efficiency of age II+ and III+ fish increased with the importance of Amphipoda to perch diet. Growth efficiency of age II+ fish decreased with the contribution of zooplankton to the diet ($F_{1,8}$ =26.18). An analysis of the effect of prey size within these taxonomic groups (Amphipoda and zooplankton) was performed. No significant influence was revealed.

The inclusion of feeding level in the list of independent variables examined revealed two new relationships for age I+ fish (Table 11b). An important departure from the previously established growth relationships was that growth efficiency decreased as feeding level increased. This result is expected given the results of previous works on growth efficiency (Paloheimo and Dickie 1966; Kerr 1971).

Relationship between Growth Efficiency and Diet Taxonomic Diversity

We found no significant relationship between growth efficiency and taxonomic diversity of the diet (age I+: $F_{1,6}$ =2.78; p >0.10; age II+: $F_{1,8}$ =0.40; p >0.50; age III+: $F_{1,8}$ =0.03; p >0.85). The inclusion of food consumption estimates in the models did not influence these results.

Relationship between Growth Efficiency and Prey Size–Classes

The relationships between growth efficiency and the contribution of various size–classes to the diet were numerous and varied (Table 11a). Growth efficiency of age I+ fish decreased exponentially as the percent contribution of size–class 1 to the diet increased ($F_{1,5}$ =8.63), but increased as the contribution of size–class 3 increased ($F_{1,6}$ =7.42). A significant quadratic relationship existed between the growth efficiency of age I+ perch and the percent contribution of size–classes 2 ($F_{2,5}$

TABLE 11. Significant relationships ($p < 0.05$) observed between (a) growth efficiency (K_1) and diet descriptors (refer to Tables 2 and 5 for the definition of prey taxonomic groups and size–classes) and (b) when food consumption estimates (ADR; Boisclair and Leggett 1989) were included in the models.

Age–classes	Models	r^2	p
(a) I+	$\ln K_1 = -0.09\ SC1 + 3.15$	0.63	0.04
	$K_1 = 0.29\ SC2^2 - 4.51\ SC2 + 31.21$	0.95	0.0005
	$K_1 = 0.60\ SC3 + 16.5$	0.55	0.03
	$K_1 = 0.44\ SC4^2 - 4.78\ SC4 + 30.22$	0.85	0.04
II+	$K_1 = -0.005\ AMP^2 + 0.50\ AMP + 4.42$	0.84	0.005
	$\ln K_1 = -0.09\ ZOO + 2.59$	0.77	0.0009
	$\ln K_1 = -0.39\ SC2 + 2.61$	0.80	0.0005
	$\ln K_1 = -0.12\ SC3 + 2.61$	0.72	0.002
	$\ln K_1 = -0.06\ SC9 + 2.39$	0.47	0.03
III+	$K_1 = -0.0025\ AMP^2 + 0.22\ AMP + 4.32$	0.65	0.04
	$\ln K_1 = -0.02\ SC7 + 2.40$	0.41	0.05
(b) I+	$K_1 = -0.005\ DIP^2 - 7.54 \ln ADR + 20.30$	0.79	0.02
	$K_1 = 0.004\ ZOO^2 - 11.16 \ln ADR + 9.27$	0.81	0.02
II+	none		
III+	none		

$= 47.88$) and 4 ($F_{2,5} = 6.89$) to the diet. The shape of these models (within the observed range of contributions of size–class 2 and 4) suggests that growth efficiency of age I+ fish increased with the importance of size–class 2 to the diet, but decreased with that of size–class 4. The growth efficiency of age II+ perch decreased exponentially as the percent contribution of size–classes 2 ($F_{1,8} = 31.06$), 3 ($F_{1,8} = 20.86$) and 9 ($F_{1,8} = 7.00$) increased. The growth efficiency of age III+ fish decreased exponentially as the percent contribution of size–class 7 increased ($F_{1,8} = 5.55$).

The combination of food consumption estimates to prey size–classes revealed no other significant relationships.

Relationship between Growth Efficiency and Mean Prey Weight

There was no significant relationship between growth efficiency and mean prey weight (age I+: ($F_{1,6} = 0.02$; $p > 0.85$; age II+: $F_{1,8} = 0.01$; $p > 0.90$; age III+: $F_{1,8} = 0.36$; $p > 0.50$). The inclusion of food consumption estimates in the models did not influence these results. The analysis of mean prey size within age–classes yielded no evidence of the existence of an optimal prey size (Fig. 5).

Relationship between Growth Efficiency and the Diversity of Size–Classes in the Diet

We found no significant relationship between growth efficiency and the size–class diversity of the diet (age I+: $F_{1,6} = 2.12$; $p > 0.15$; age II+: $F_{1,8} = 4.81$; $p > 0.05$; age III+: $F_{1,8} = 1.05$; $p > 0.30$). The inclusion of food consumption estimates in the models did not alter this result.

General Patterns between Growth Rates and Diet Descriptors

For most of the relationships listed in Table 10, it was possible to determine whether growth rates increased cr decreased relative to the contribution of a given prey type to the diet. This was done by noting the sign of its coefficient in the model. For the quadratic models, we plotted the growth rates predicted from the calculated relationships using as input values percent prey contributions within the range of observed values, and then noted the general trend. When classified by the sign and the magnitude of their coefficients, the prey descriptors that explained a significant proportion of among-population variability of age I+ and II+ fish growth rates displayed a clear pattern (Fig. 6). Too few significant relationships were observed to perform such an analysis for age III+ fish. This analysis suggests that growth increased with the percent contribution of intermediate prey sizes as assumed by optimal foraging models.

The growth rates of age I+ and II+ perch decreased with increasing contributions of both small and large prey to their diet. Growth rates increased as the percent contribution of prey of intermediate sizes in the diet increased. While many of the diet descriptors used in this analysis are interdependent (zooplankton and prey of size–classes 2 and 3), the analysis does suggest that prey of intermediate sizes (approximately 1 mg dry weight) were optimal for the growth rates of age I+ and II+ perch (Fig. 6). However, when the relationship between fish growth rates and our estimates of mean prey weight were analysed directly, there was no significant relationship.

General Patterns between Growth Efficiency and Diet Descriptors

When classified by the sign of their coefficient in the models linking growth efficiency to diet (Table 11), these diet descriptors previously identified as significant contributors to the among-population variability in growth efficiency, revealed a pattern similar to that displayed by the growth relationships (Fig. 6). Growth efficiency decreased as the contribution of small and large prey increased, and increased with the contribution of intermediate-size prey to the diet. This qualitative analysis suggests that prey of approximately 0.015 mg dry weight and 1 mg dry weight were, respectively optimal for the growth efficiency of age I+ and II+ perch (Fig. 6). However, as with the analysis of growth rates, there was no evidence of this pattern when the relationship between fish growth efficiency and our estimates of mean prey weight were analysed directly.

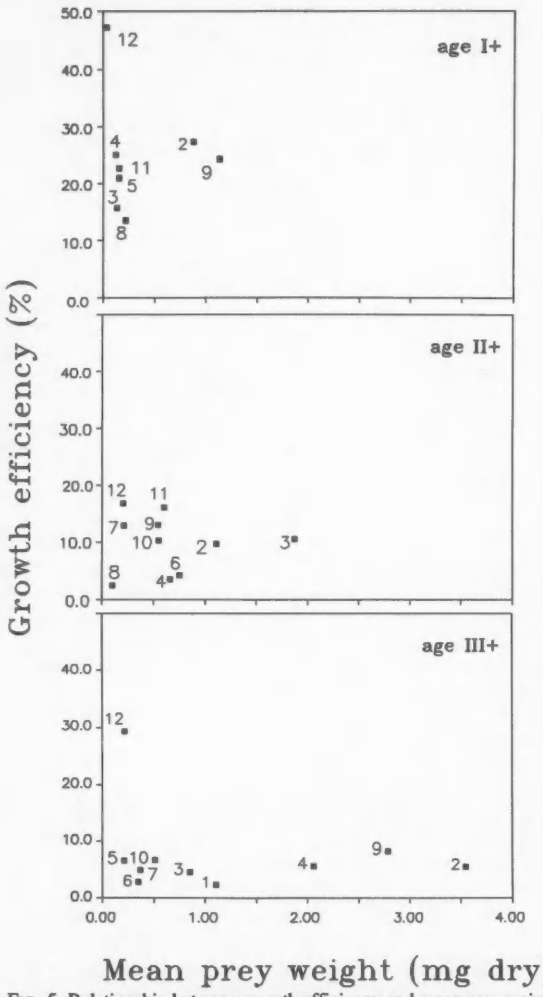

FIG. 5. Relationship between growth efficiency and mean prey weight for each age–class studied. The populations are identified using the legend in Fig. 1.

In the analyses described above we considered all relationships significant at $p < 0.05$. We did so because of the exploratory nature of this work. However, caution should be exercized in interpreting or using the individual models presented. Given the number of relationships examined, some significant relationships are likely to have occurred by chance. A more rigorous significance level (estimated using the Bonferroni method for multiple tests, Cooper 1968; Miller 1977; Oden 1984) would be 0.0017. The adoption of this more rigorous standard diminishes the total number of statistically significant relationships observed (Table 10 and 11) but does not alter the general conclusions we draw from the analysis.

Discussion

The most extensively developed hypothesis regarding the influence of prey type on fish growth involves prey size. This hypothesis predicts that both fish growth rates and growth efficiency initially increase with prey size but reach an optimal level at intermediate prey sizes. Our analysis of the sign of the coefficients associated with diet descriptors that explained a significant proportion of the variability in growth rates and/or growth efficiency among the populations we studied supports this hypothesis. However, direct analyses of the relationship between these parameters and mean prey weight within age–classes revealed no such pattern. Contrary to the assumption of optimal foraging theory, many of the fish populations we studied exhibited higher than average growth rates despite the small mean weight of the prey they consumed. Hence our analyses of the relationship between in situ interpopulation differences in growth rates and mean prey weight do not reveal a general pattern for the influence of prey size on fish growth.

The difficulty of deriving a general relationship between in situ fish growth and prey size is undoubtedly related to the influence of the multitude of interacting factors inherent in field situations (Weatherley 1972; Brett 1979; Brett and Groves 1979). This is likely to be especially critical when dealing with a relatively large number of populations, covering a wide range of environmental characteristics. Furthermore, the relative importance of the factors that interact to produce or mask a general relationship between in situ fish growth and prey size varies depending on the type of relationship examined.

Among-Population Relationships between Prey Type and Growth

The advantage of a given prey type is hypothesized to lie in a lower energetic cost/benefit ratio of foraging. The foraging cost of a given prey type is believed to be related to two distinct components; the search for, and the handling of, that prey (Werner and Hall 1976; English 1983; Stevens and Krebs 1986). The handling component of foraging cost is often described as a function of prey size (Werner and Hall 1974; Kislalioglu and Gibson 1976a; Gillen et al. 1981; Mittelbach 1981, 1983; Hoyle and Keast 1987). The most eloquent experiments supporting the hypothesis that a strong relationship should exist between growth and mean prey weight were executed in the laboratory under conditions of near constant prey abundance/availability. Hence, the results are representative of conditions when the searching component of foraging cost was relatively constant (Nakamura and Kasahara 1956; Prather 1958; Wankowski 1979; Wankowski and Thorpe 1979; Hart and Connellan 1984). A more limited number of in situ studies (comparative and experimental) have yielded similar findings (Mills and Forney 1981; Nyberg 1979; English 1983; Frank and Leggett 1986).

The searching component is believed to be directly influenced by prey abundance/availability (Schoener 1969; 1971; Ware 1972, 1973; Norberg 1977; Werner et al. 1981). Mean prey weight is thus an adequate measure of among-populations differences in average handling cost but cannot account for searching costs. In nature, both handling costs (defined by prey size) and searching costs (defined by prey abundance/availability) operate. As our work illustrates, it is unlikely that a clear pattern will exist between in situ growth and mean prey weight alone, since this parameter does not adequately account for prey abundance/availability. In our study, prey abundance/availability varied among lakes (Rasmussen 1988a).

In contrast to mean prey weight, the percent contribution of a given prey type to the diet integrates the interaction between the handling and searching component of foraging cost. The percent contribution of a given prey type to the diet is known to be influenced by prey abundance/availability (Ware 1972,

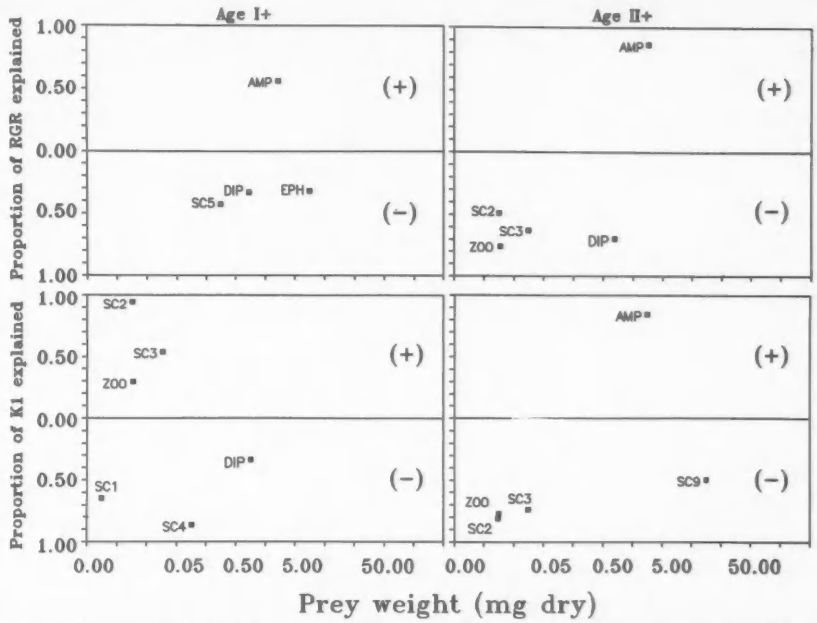

FIG. 6. Proportion of variability in among-population growth rates (RGR) and growth efficiency (K_1) exhibited by age I+ and age II+ fish that was explained by diet descriptors. The descriptors are classified according to the sign of their coefficient in the models presented in Table 10 and 11. Growth (and growth efficiency) generally increased with the percent contribution to the diet of prey types having a positive coefficient (upper panel of each graph) and decreased with the percent contribution to the diet of prey types having a negative coefficient (lower panel of each graph). Note that the prey weight scale is not linear, but corresponds to the scale used to define prey size–classes (Table 2).

1973; Werner et al. 1981; Mittelbach 1981, 1983). Moreover, most of the prey taxonomic groups consumed by the fish we sampled represented a very limited size spectrum (and hence few size–classes; Table 2). We thus hypothesized that relationships would be more evident between growth and the percent contribution of a given prey type than between growth and mean prey weight.

This expectation was supported by our results and is also supported by the results of the limited number of previous studies that have attempted to explain among-lake variability of fish growth in situ. Martin (1966) analysed 30 populations of lake trout (*Salvelinus namaycush*) in Algonquin Park (Ontario). He found a significant negative relationship between average fork length at age 7 and the percent contribution of zooplankton to the diet ($r^2 = 0.42$). We reanalyzed Martin's data to verify that this correlation was not due to a relationship between fish size and diet. We used (fish length at age 8 − length at age 6)/2 as a measure of growth rate at age 7. This measure of growth was not significantly related to fish length at age 7 ($F_{1,28} = 1.47$; $p > 0.20$). It was, however, negatively related to the percent contribution of zooplankton to the diet ($F_{1,28} = 8.97$; $p < 0.01$). This confirms Martin's interpretation. By our analysis the contribution of zooplankton to the diet explained 24% of among-population variability of trout growth rates.

To our knowledge, the only other in situ study to directly address the influence of prey type on interpopulation growth is that of Hansson (1985). He assessed perch growth at 10 sites of the Baltic archipelago, and reported that perch growth rates appeared to decrease as the proportion of zooplankton in the diet increased. However, the relationship was not significant. In both the Martin and the Hansson studies growth was most strongly related to the percent contribution of a given prey type in the diet. There was no relationship between growth and mean prey weight. The results of our study are consistent with, and support those of Martin and Hansson. Moreover, our study emphasizes the point that mean prey weight does not provide a consistent explanation of in situ variability of fish growth even when combined with estimates of the quantity of food consumed.

Within Age–Class Relationships between Prey Type and Growth

Our analysis of the relationship between growth rates and specific prey taxonomic groups or size–classes further supports this conclusion. For example, the sign of the relationships between growth rates or growth efficiency and the percent contribution of Amphipoda was positive for both age–classes I+ and II+. However, for these same age–classes the sign of these relationships when analysed for Diptera was negative in spite of the fact that these prey have similar mean sizes (Table 5). These differences may be related to differences in the visibility, the abundance or the nutritive value (Cowey and Sargent 1979; Tacon and Cowey 1985) of Amphipoda and Diptera (predominately Chironomidae).

In many other cases we observed similar relationships between growth rates (and/or growth efficiency) and prey of very different sizes. Growth efficiency of age I+ fish decreased as the percent contributions of size–class 1 and Diptera (size–classes 5–7) to the diet increased. The growth rates and growth

efficiency of age II+ perch decreased as the percent contribution of zooplankton (size–classes 1–3) and prey of size–class 9 in the diet increased. The extent to which the patterns observed are related to prey size or to among-lake variability in prey abundance is presently under analysis (Rasmussen and Boisclair, unpubl. data).

Among Age–Class Relationships between Prey Type and Growth

Among age–class comparisons focus more directly on the influence of prey size on growth. The growth of age I+ fish was positively correlated with the percent contribution of zooplankton and prey of size–classes 2 and 3 in the diet. In contrast, both the growth, and the growth efficiency, of the larger age II+ fish tended to decrease as the percent contribution of zooplankton in the diet increased. Since age I+ and II+ fish were surveyed simultaneously, and in the same lakes, we rejected the possibility that differences in the quantity (biomass) of zooplankton available to these age–classes was responsible for the opposite signs of the regression coefficients. The hypothesis that younger/smaller perch graze more efficiently on zooplankton than older/larger fish is more tenable (Noble 1975; Nyberg 1979; Hansson 1985). Conversely, older/larger perch should graze more efficiently on larger preys. These hypotheses are supported by our findings. Maximum growth efficiency of age II+ fish occurred when they consumed organisms averaging 1.0 mg (dry weight). The equivalent prey size for age I+ fish was 0.015 mg. Mousseau (1983) reported that the growth of the slimy sculpin (*Cottus cognatus*) in three Ontario lakes could be ranked as a function of the biomass of large prey items (>100 μg) available to adults and of small prey items (<100 μg) available to juveniles.

Relationship between Fish Weight and Diet Diversity

The results of our studies are inconsistent with predictions of optimal foraging theory as they concern diet diversity. Werner (1979) used prey profitability functions to show that older and/or larger fish of a given species are, from a foraging energetics perspective, less influenced by prey size than younger and/or smaller fish. He further inferred that the diet diversity of large fish should be higher than that of smaller fish. Mittelbach (1981), using a similar approach, found that the net rate of energy intake of small fish was influenced very little by prey size. This implies that the diet diversity of small fish should be higher than for larger fish.

The apparent contradiction of these predictions probably results from experiments conducted with different prey communities (G. G. Mittelbach, Kellogg Biological Station, Michigan State University, pers. comm.). Our analyses do not conform to either prediction. Diet diversity (measured taxonomically or by size–classes) did not vary significantly either among size–classes or with fish size.

Among-lake differences in prey community structure are likely to operate on prey selection via the influence of prey motion (Zaret 1979; Eggers 1982), morphology (Zaret 1975; Zaret and Kerfoot 1975), behavior (Vinyard 1980, 1982; Moore and Moore 1976), color (Ginetz and Larkin 1973; Kislalioglu and Gibson 1976b), contrast (Ware 1973; English 1983), and nutritive value (Cowey and Sargent 1979; Tacon and Cowey 1985). Many workers have also documented the influence of water transparency (Zaret 1979; Moore and Moore 1976), light (Vinyard and O'Brien 1976), habitat complexity (Ware 1972, 1973; Nelson 1979), the presence of other fish species (Werner and Hall 1974, 1976; Werner et al. 1983; Schmitt and Coyer 1983; Hanson and Leggett 1985, 1986; Persson 1983, 1986) on prey selection by fish. Among-lake variability of one or more of these factors is likely to cause prey profitability functions derived from cost/benefit relationships based on prey size to be environment specific. Such models, while useful as an aid to the understanding of processes which may influence growth (niche shifts, habitat use, and fish interactions; Werner and Hall 1976, 1979; Werner 1979; Mittelbach 1981; Werner et al. 1981; Werner and Gilliam 1984) or the prediction of growth within habitats are unlikely to be useful in explaining interpopulation differences in in situ growth rates or growth efficiency.

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Some Procedures for use in Cohort Analysis and Other Population Simulations

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The catch equation $N_{t+1} = N_t e^{-(F_t+M)}$ must often be solved either forwards or backwards. Because F_t is an implicit function of catch, population size, and M, the solution must be either iterative or approximate. In this paper an improved approximation to N_{t+1} is developed of the form

$$N_{t+1} = N_t exp(-M) - C_t exp(-AM)$$

for the forward solution and a corresponding equation for backward solution. Appropriate values of A are presented for a range of combinations of M and F; and $A = 0.585$ gives approximations to N_{t+1} with an error of less than 1% provided that (F_t+M) is less than about 1.5. Much closer approximations are obtained if A is calculated for each case as a polynomial in M and either C_t/N_t for the forward solution or C_{t-1}/N_t for the backward solution. The appropriate coefficients for these polynomials are derived both by truncating Taylor's expansion and by statistical methods, and are presented here. One such polynomial for A gives errors of less than 0.02% where $(F+M)$ is less than 1.1. Similarly good approximations can also be obtained by interpolating into a table of N_{t+1}/N_t against M and C/N.

L'équation de la prise $N_{t+1} = N_t e^{-(F_t+M)}$ doit souvent être extrapolée ou intrapolée. Parce que F_t est une fonction implicite de la prise, de l'importance de la population et de M, la solution doit être itérative ou approximative. Dans cet article, une approximation améliorée de N_{t+1} est proposée; elle prend la forme suivante

$$N_{t+1} = N_t exp(-M) - C_t exp(-AM)$$

pour l'extrapolation et il existe une équation correspondante pour l'intrapolation. Des valeurs appropriées de A sont présentées qui correspondent à différentes combinaisons de M et F; $A = 0{,}585$ donne des approximations de N_{t+1} avec une erreur de plus ou moins 1 % pourvu que (F_t+M) soit inférieur à environ 1,5. Des approximations beaucoup plus précises sont obtenues lorsque A est calculé dans chaque cas comme un polynome de M et C_t/N_t pour l'extrapolation ou $C_{t-1}N_t$ pour l'intrapolation. Les coefficients appropriés à ces polynomes sont déduits à la fois en tronquant l'expansion de Taylor et par des méthodes statistiques; ils sont présentés dans cet article. Un tel polynome pour A produit une erreur de moins de 0,02 % quand $(F+M)$ est inférieur à 1,1. De la même façon, on peut obtenir de bonnes approximations en faisant une interpolation suivant une table de N_{t+1}/N_t contre M et C/N.

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Several techniques used in the analysis of exploited populations of marine animals involve calculation of the population of one year from the population at the beginning of an adjacent year, knowing the catch taken in the first year and knowing, or assuming, the natural mortality rate.

In cohort analysis (Gulland 1977) the calculation is performed backwards; the population at the beginning of one year is calculated from that at the beginning of the following year. In other cases, the calculations are done forward (Allen 1983; Hampton and Majkowski 1986). In the following discussion the forward simulation is treated first because it is rather more direct, although in practice the backward solution, employed in cohort analysis and VPA is probably more widely used.

In the most usual population model, when catching is continuous, and both fishing (F_t) and natural (M) mortality rates remain constant, the basic equations are

$$(1) \quad C_t = F_t N_t (1 - exp(-(F_t + M)))/(F_t + M),$$

and

$$(2) \quad N_{t+1} = N_t exp(-(F_t + M)),$$

where N_t is the population at the beginning of year t and C_t is the catch in the same year. Equations (1) and (2) are solved for N_{t+1} and F_t in terms of N_t and C_t in forward simulation, and for N_{t-1} and F_{t-1} in terms of N_t and C_{t-1} in the backward case.

Equation (1) cannot be solved analytically; an iterative solution is required. It can be solved, for example, by Newton's

TABLE 1. Values of A for M = 0.1–1.0, and F equal to 0.1 and to the value of M.

	A	
M	$F = 0.1$	$F = M$
0.1	0.512	0.512
0.2	0.517	0.525
0.3	0.521	0.537
0.4	0.525	0.550
0.5	0.529	0.562
0.6	0.533	0.574
0.7	0.537	0.586
0.8	0.541	0.597
0.9	0.545	0.609
1.0	0.550	0.620

method, or by the method proposed by MacCall (1986). While such a solution can now be obtained with a computer, or even with some desk calculators, the number of calculations required may be substantial. For a typical cohort analysis, for example, this number will be approximately the product of the number of age–groups and the number of years for which there are data. If higher level iterations are required (e.g. to test the effect of different values of M or of the terminal F), then the number of calculations needed will be increased by a further factor. A substantial amount of computer time may therefore often be saved by calculating an approximation to N_{t+1} or N_{t-1} directly, using an algorithm that gives an acceptable level of accuracy. An approximation frequently used for this purpose (Ricker 1975) is

$$(3)\quad N_{t+1} = N_t\, exp(-M) - C_t\, exp\,(-M/2),$$

which Pope (1972) derived as an approximation to equations (1) and (2) when combined as

$$(4)\quad N_{t+1} = N_t\, exp(-M) - BC_t\, exp(-M),$$

where

$$(5)\quad B = \frac{(F_t+M)\,(1-exp(-F_t))}{F_t(1-exp(-(F_t+M))}$$

MacCall (1986) discussed the merits of simplifying cohort analysis formulae and proposed an approximation that is slightly more accurate than equation (3). Sims (1982) has also proposed approximations to N_{t+1}/N_t and N_{t-1}/N_t which are much better than those given by Pope (1972).

Two different approaches are here used to develop approximations to B (in terms of M and C_t/N_t) that estimate N_{t+1} much more accurately than does equation (3). A third approach also presented is to obtain an approximation to N_{t+1}/N_t in terms of M and C_t/N_t. These approaches are also used to develop approximations to the "backward" solution.

Methods

Equation (4) can be rewritten exactly as

$$(6)\quad N_{t+1} = N_t\, exp(-M) - C_t\, exp(-AM),$$

where

$$(7)\quad A = 1 - \ln B/M.$$

Table 1 and Appendix 1 show the values of A for combinations of a range of values of F_t and M. Pope's (1972) approximation is a version of equation (6), which sets $A = 0.5$. It is evident that values of A higher than 0.5 will produce better approximations to N_{t+1}.

Gray (1979) pointed out that the expression $exp(-x/2.25)$ is an approximation to $(1-exp(-x))/x$ with an error of less than 2%, over the range for x of 0 – 1.6. Substituting F_t+M, and F_t for x in equation (5) shows that this is equivalent to giving A in equation (6) a value of 0.556, which is consistent with the values in Appendix 1.

Development of Approximations

In practical applications the basic data available are the catch in a given year, the population at the beginning or end of that year, and a value for M for that year. From these, we have for each year C_t/N_t, or C_{t-1}/N_t and M, and it is therefore useful to consider obtaining approximations in which A or B is replaced in equations (7) and (5) by expressions in M and C_t/N_t.

Taylor's Approximation

One way of calculating approximation formulae is to use a Taylor's series. As a starting point take an expression for the approximation for B of the form

$$(8)\quad B^* = exp\left(\frac{M}{2}(1 - aM - bC_t/N_t)\right)$$

and determine a and b by matching terms in Taylor's expansions for B and B^*.

From the definition of B in equation (5), and since

$$(1 - exp(-x))/x = 1 - x/2 + x^2/6 \ldots.,$$

we can write

$$(9)\quad B = \frac{(1-F_t/2 + F_t^2/6\ldots)}{(1 - (F_t+M)/2 + (F_t+M)^2/6\ldots)}$$

$$(10)\quad = 1 + \frac{M}{2} + M^2/12 - F_tM/12\ldots.$$

If F_t and M are small then from equation (1)

$$(11)\quad F_t \doteq C_t/N_t,$$

and therefore

$$(12)\quad B \doteq 1 + \frac{M}{2} + M^2/12 - M(C_t/N_t)/12.$$

Also, since the Taylor's expansion of equation (8) is

$$(13)\quad B^* = 1 + M/2 - \left(\frac{a}{2} - \frac{1}{8}\right)M^2 - \frac{b}{2}MC_t/N_t,$$

matching terms in equations (12) and (13) results in

$$a = 1/12$$
$$b = 1/6,$$

and substituting into equation (8) gives

$$(14)\quad B^* = \exp\left(\frac{M}{2}(1 - M/12 - (C_t/N_t)/6)\right).$$

TABLE 2. Coefficients of regression equations to calculate A^* from M and C_t/N_t values for both forward (F) and backward (B) simulation.

No. of variables	Forward/ backward	Coefficients for A^*					
		b_0	b_1	b_2	b_3	b_4	b_5
		Coefficients					
		Constant	M	M^2	C_t/N_t	$(C_t/N_t)^2$	MC_t/N_t
4	F	0.466284	0.080881	−0.010352	0.194764	—	—
6	F	0.510654	0.031815	0.000489	0.000193	0.181930	0.104462
4	B	0.536366	0.023820	−0.011794	0.020526	—	—
6	B	0.517744	0.021340	0.005853	0.039684	−0.002796	−0.005996

Over the range $0 < M \leq 0.3$, $0 < F \leq 1.2$ the absolute error in B^* as an approximation to B is less than 2%. For this range, the resulting errors in N_{t+1} are less than half those from Pope's (1972) approximation $A = 0.5$, and also much less than those given by the improved expression of McCall (1986).

Multiple Regression Approximations

More accurate approximations could be obtained by taking further terms in the Taylor's expansion of B, but several terms would be required for a high accuracy. However, empirical development of approximations of similar general form has been found to give very satisfactory results.

Good approximations can be obtained by fitting coefficients for the parameters of the formulae by multiple regression on the calculated values of A over a grid of values of M and F_t. This has been done for two expressions:

$$(15)\quad A^* = b_0 + b_1M + b_2M^2 + b_3C_t/N_t$$

and

$$(16)\quad A^* = b_0 + b_1M + b_2M^2 + b_3C_t/N_t + b_4(C_t/N_t)^2 + b_5MC_t/N_t.$$

Table 2 presents the coefficients for both expressions for A^*, which were obtained by fitting the approximation to exact values of A on a 10 × 10 grid with $M = 0.1 - 1.0$ and $F = 0.15 - 1.5$.

In backward simulation, as required in cohort analysis, the basic equation corresponding to equation (6) is:

$$(17)\quad N_{t-1} = N_t\, exp(M) + C_{t-1}\, exp(M\,(1-A)),$$

where A has the same values as in the forward extrapolation. Approximations for N_{t-1} can be obtained as before either from the Taylor's expansion or by regression. Since the known quantity that has to be used for F_{t-1} is now C_{t-1}/N_t, instead of C_t/N_t, the coefficients are different. They are shown in Table 2.

In the third approach mentioned above, the ratio N_{t+1}/N_t is obtained directly from C_t/N_t and M by solving equation (1) for F_t in terms of C_t/N_t and M, and substituting in equation (2); an equivalent procedure is followed to calculate N_{t-1}/N_t in terms of C_{t-1}/N_t and M. Tables for these ratios for both forward and backward calculations are given in Appendix 2.

Discussion

When M and F are both small (e.g. less than 0.2) there is no difficulty in obtaining a good approximation to N_{t+1}/N_t or its backward equivalent. The Pope (1972) approximation is probably adequate for many purposes, and that proposed by Sims (1982) is excellent. It is when M and F are higher, particularly when $F+M > 1.0$, that the problems arise, and it is in this situation that the procedures discussed in this paper are superior to the approximations described earlier. Even the less accurate approximations can be used, as Sims (1982) suggests, to give starting values for an iterative procedure, but much time can be saved by using approximations which are good enough to make this unnecessary.

The procedures discussed in this paper can be used to obtain an approximation to the desired population size in several different ways. Two of these involve calculating N_{t+1} by equation (6) or its equivalent for N_{t-1}, These are:

(1) to use an appropriate constant value of A throughout the series;

(ii) to calculate an individual value of A for each cell for a year–class and season from the initial population and catch sizes using one of the approximations for A.

It would also be possible to obtain annual values of A by interpolating in a table of this parameter against M and C/N. However, if interpolation is to be used it is more direct, faster, and little less accurate,

(iii) to calculate N_{t+1} or N_{t-1} by interpolating for the appropriate ratio of N_{t+1}/N_t or N_{t-1}/N_t in tables of these ratios against M and C_t/N_t or C_{t-1}/N_t.

In all these cases an appropriate value of M has to be provided for each estimation.

In simulation or cohort analysis it may sometimes be convenient to use the multiplicative expression.

$$(18)\quad N_t = N_t\, exp(-M) - C_t D\, exp\,(-M/2)$$

instead of the exponential algorithms based on equation (6). This equation may have some advantage if the model were to be subjected to further mathematical treatment, for example by differentiating with respect to M. If it is desired to do this, D may be calculated by using the relationship $D = exp(M(0.5 - A))$.

(a) Single Value of A

This is the simplest procedure and is appropriate when M can reasonably be taken as constant, and when the rate of exploitation does not vary greatly over the period of the study. It will provide a simple and rapid way of carrying out a preliminary analysis. Table 1 would provide a useful guide in selecting the preliminary value of A for a given M. In general the values corresponding to $F = 0.1$ might be appropriate for a lightly exploited stock, or those for $F = M$ if exploitation is fairly heavy. If more specific estimates of F and M are available, appropriate values can be obtained from Appendix 1. There are analytic advantages in using a single value of A in equations

TABLE 3. Percentage error in the value of N_{t+1} or N_{t-1} using the simple expression with $A = 0.55$; calculated over a grid with $M = 0.05$–0.5 and $F = 0.05$–0.5.

	Forward *F*									
M	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
0.05	0.01	0.02	0.03	0.03	0.04	0.04	0.04	0.04	0.03	0.02
0.10	0.02	0.04	0.05	0.06	0.07	0.07	0.07	0.06	0.05	0.03
0.15	0.03	0.06	0.08	0.09	0.10	0.10	0.09	0.08	0.05	0.02
0.20	0.04	0.07	0.09	0.11	0.12	0.12	0.11	0.08	0.05	0.00
0.25	0.05	0.08	0.11	0.13	0.13	0.13	0.11	0.08	0.03	−0.03
0.30	0.05	0.09	0.12	0.14	0.14	0.13	0.11	0.07	0.01	−0.07
0.35	0.06	0.10	0.13	0.15	0.15	0.13	0.09	0.04	−0.03	−0.13
0.40	0.06	0.10	0.13	0.15	0.14	0.12	0.07	0.01	−0.09	−0.20
0.45	0.06	0.11	0.14	0.15	0.14	0.10	0.04	−0.04	−0.15	−0.29
0.50	0.06	0.11	0.14	0.14	0.12	0.08	0.01	−0.09	−0.22	−0.39
	Backward *F*									
0.05	−0.01	−0.02	−0.02	−0.03	−0.03	−0.03	−0.03	−0.02	−0.02	−0.01
0.10	−0.02	−0.04	−0.05	−0.05	−0.06	−0.05	−0.05	−0.04	−0.03	−0.02
0.15	−0.03	−0.05	−0.07	−0.07	−0.08	−0.07	−0.07	−0.05	−0.04	−0.01
0.20	−0.04	−0.06	−0.08	−0.09	−0.09	−0.09	−0.07	−0.06	−0.03	0.00
0.25	−0.04	−0.07	−0.09	−0.10	−0.10	−0.10	−0.08	−0.05	−0.02	0.02
0.30	−0.05	−0.08	−0.10	−0.11	−0.11	−0.10	−0.08	−0.04	0.00	0.04
0.35	−0.05	−0.09	−0.11	−0.12	−0.11	−0.10	−0.07	−0.03	0.02	0.08
0.40	−0.06	−0.09	−0.12	−0.12	−0.11	−0.09	−0.05	0.00	0.05	0.12
0.45	−0.06	−0.10	−0.12	−0.12	−0.11	−0.08	−0.03	0.03	0.09	0.17
0.50	−0.06	−0.10	−0.12	−0.11	−0.09	−0.06	−0.01	0.06	0.14	0.23

TABLE 4. Mean and root mean square (RMS) percentage errors in predicted values of N_{t+1} or N_{t-1} from four-term and six-term formulae for A^* for forward (F) and backward (B) simulations calculated over a grid of $M = 0.1 - 1.0$ and $F = 0.15 - 1.5$, and from interpolation into tables of N_{t+1}/N_t calculated over 1000 random points within the same range of F and M.

No. of variables	Forward/ Backward	Mean error	RMS error
	*Algorithms for A**		
4	F	−0.1760	0.833
6	F	−0.0086	0.121
4	B	0.0087	0.398
6	B	0.0012	0.125
	Interpolation for N_{t+1}/N_t		
	F	−0.006	0.16
	B	0.001	0.02

(6) and (17), because these equations will then be linear in C/N.

If the exploitation rate has varied extensively or M changes (e.g. as a result of age-dependency), it may be useful to use values of A which minimize average errors over a range of combinations of F and M. Over a range of M and F between 0.1 and 1.0, $A = 0.585$ minimizes the average root mean square (RMS) error. The RMS errors in N_{t+1} and N_{t-1} are 1.3 and 0.55% for forward and backward runs, respectively.

If the likely values for M and F are less than above, the corresponding values of A will be smaller. Over a range of M and F up to 0.5 the average RMS error is least with $A = 0.55$ being 0.10% for forward calculation, and 0.08% for backward calculation. The full error grid for forward simulation with $A = 0.55$ is shown in Table 3.

It is important to remember that in backward calculations the exponent is $(1-A)$, not A.

Tests have also shown that where M is fairly low (e.g. about 0.2) and the true F_t does not vary from year to year by more than about ±20% of the mean, use of the simple equation (6) with A appropriate to the mean F_t will yield approximations about as good as those obtained by using the six-coefficient polynomial in equation (16).

(b) Individual Values of A — Calculated

Use of individual values of A provides good estimates of the next population size even when M and the exploitation rate are changing. This can be done simply and rapidly by including one of the polynomial formulae, with the appropriate values for the coefficients, in a computer program.

Table 4 summarises the error levels given by these formulae over a grid of $M = 0.1 - 1.0$ and $F = 0.15 - 1.5$. As an example, the detailed error matrix for the six - coefficient forward formula for A is shown in Table 5. The arithmetic mean error is −0.008% and the RMS error is 0.12%, but this is raised by the comparatively high errors for $F = 1.5$. This table shows a pattern of distribution which results from the nature of the polynomial surface; errors are very small when M and F are small (less than 0.03% when F is less than 0.5), but they become relatively large, with a complex distribution pattern, towards the lower right corner of the table. Within a grid of $M = 0.1 - 1.0$, $F = 0.1 - 1.0$ the same coefficients give an RMS error of 0.050% and a mean error of 0.017%. For the backward calculations the accuracy of the A algorithm is close to that for the forward calculations.

TABLE 5. Percentage error in the value of N_{t+1} using the six-term formula in A^* with the coefficients given for forward simulation in Table 2.

	F									
M	0.15	0.3	0.45	0.6	0.75	0.9	1.05	1.2	1.35	1.5
0.1	0.00	−0.01	−0.01	−0.01	0.00	0.01	0.03	0.03	0.02	−0.02
0.2	0.01	−0.01	−0.02	−0.02	0.00	0.02	0.05	0.05	0.02	−0.08
0.3	0.01	−0.01	−0.02	−0.02	0.00	0.04	0.06	0.06	0.01	−0.15
0.4	0.01	−0.01	−0.02	−0.01	0.02	0.05	0.08	0.08	−0.01	−0.22
0.5	0.01	−0.01	−0.02	0.00	0.03	0.08	0.11	0.09	−0.02	−0.28
0.6	0.01	0.00	−0.01	0.01	0.05	0.11	0.14	0.12	−0.01	−0.31
0.7	0.01	0.00	0.00	0.03	0.08	0.14	0.18	0.16	0.01	−0.32
0.8	0.01	0.00	0.01	0.04	0.11	0.18	0.23	0.22	0.07	−0.29
0.9	0.01	0.00	0.01	0.06	0.14	0.24	0.30	0.29	0.15	−0.21
1.0	0.00	0.00	0.02	0.09	0.19	0.30	0.38	0.40	0.27	−0.08

(c) Direct Interpolation for N_{t+1}/N_t

The level of accuracy will depend on the number of digits stored and the spacing of the points. The tables in Appendix 2 show N_{t+1}/N_t or N_{t-1}/N_t to four decimal places over the ranges $M = 0.1$–1.0, $C_t/N_t = 0$–0.9 and $C_{t-1}/N_t = 0$–3.0. The errors from interpolation in this table are consistently positive so that the average RMS error can be reduced by subtracting a correction factor from the linearly interpolated value.

Appropriate correction factors are 0.27% for forward simulation and 0.04% for backward simulation. After these corrections are applied the RMS errors are about 0.16 and 0.023%, respectively. There is no advantage in using an interpolation table with a large number of digits.

Using a nonlinear interpolation procedure will also increase the level of accuracy and remove much of the positive bias so that a correction factor is not necessary. The extent of the improvement will obviously depend on the routine used.

The level of accuracy can also be increased by reducing the intervals in the interpolation table. If these are halved to 0.05 and 0.15 for M and C/N, respectively, the RMS errors are reduced to about 0.021 and 0.0087% for forward and back simulations, respectively. The bias is also reduced; for the forward operation a correction factor of 0.0465% is appropriate and has been applied to obtain the errors given above; for the backward calculation no correction is necessary to reduce the RMS errors below 0.01%.

Where M is constant over a large number of calculations, a fast and accurate procedure would be to set up initially a vector of N_{t+1}/N_t against C_t/N_t for this value of M, and interpolate in this as needed.

The errors for the interpolation procedure are included in Table 4 for comparison with those for the four- and six- coefficient algorithms. They are given with less precision than those for the formula since, for obvious reasons, the means are calculated over a few thousand random points, rather than for the fixed grid used for the calculated values.

Exact comparison between the size of the errors produced with the calculated expressions and with interpolations has little meaning for two reasons. One is that the calculated values are reproducible, being based on a fixed uniformly distributed grid of points, while the interpolation points are randomized. The second and more important reason is that the calculated errors are distributed over the field in a nonuniform way depending on the shape of the polynomial surface, and having high and low error regions, while the interpolated errors are uniform over the field as a whole and vary only within the individual cells of the table.

Choice of Method

Choice of the method to be used will generally depend on the degree of analytic simplicity and the level of accuracy desired, the importance of speed, and the computer facilities available. The simplest and fastest method is to use the A algorithm with a single value appropriate to the data. When greater accuracy is wanted parameters should be adjusted for each cell and the choice is between calculating A values each time or using interpolation methods. If it is impossible (e.g. on many desk calculators) or otherwise not desirable to store an interpolation table then calculation methods must be used.

Where available the interpolation methods will generally be preferable. Not only will they probably be faster, but they also give almost uniform accuracy over the whole field of parameter values to be used, whereas the calculated values are highly accurate over part of the field but lose accuracy rapidly as the upper margins are approached. It seems therefore that, where all procedures are possible, interpolation in a table of N_{t+1}/N_t (or N_{t-1}/N_t), as in Appendix 2, should be the method of choice.

Whatever the method used, the errors introduced by any of the approximation procedures described here are likely to be small in most cases compared with the uncertainties introduced from sampling and other errors in the original data.

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APPENDIX 1. Values of A for $M = 0.1–1.0$; $F = 0.1–1.0$.

	F									
M	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
0.1	0.512	0.521	0.529	0.537	0.546	0.554	0.562	0.570	0.578	0.586
0.2	0.517	0.525	0.533	0.541	0.550	0.558	0.566	0.574	0.582	0.590
0.3	0.521	0.529	0.537	0.546	0.554	0.562	0.570	0.578	0.586	0.594
0.4	0.525	0.533	0.541	0.550	0.558	0.566	0.574	0.582	0.590	0.598
0.5	0.529	0.537	0.546	0.554	0.562	0.570	0.578	0.586	0.594	0.601
0.6	0.533	0.541	0.550	0.558	0.566	0.574	0.582	0.590	0.598	0.605
0.7	0.537	0.546	0.554	0.562	0.570	0.578	0.586	0.594	0.601	0.609
0.8	0.541	0.550	0.558	0.566	0.574	0.582	0.590	0.597	0.605	0.613
0.9	0.545	0.554	0.562	0.570	0.578	0.586	0.593	0.601	0.609	0.616
1.0	0.550	0.558	0.566	0.574	0.582	0.590	0.597	0.605	0.613	0.620

APPENDIX 2. (A) Forward simulation; values of N_{t+1}/N_t against M and C_t/N_t.

	C/N									
M	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0.1	0.9048	0.8098	0.7150	0.6205	0.5262	0.4323	0.3389	0.2462	0.1547	0.0657
0.2	0.8187	0.7286	0.6388	0.5495	0.4608	0.3729	0.2859	0.2005	0.1176	0.0403
0.3	0.7408	0.6553	0.5704	0.4862	0.4029	0.3208	0.2402	0.1619	0.0876	0.0226
0.4	0.6703	0.5893	0.5091	0.4299	0.3518	0.2753	0.2009	0.1296	0.0639	0.0115
0.5	0.6065	0.5299	0.4542	0.3797	0.3067	0.2356	0.1672	0.1028	0.0455	0.0053
0.6	0.5488	0.4763	0.4050	0.3351	0.2670	0.2011	0.1385	0.0808	0.0317	0.0022
0.7	0.4966	0.4281	0.3610	0.2955	0.2320	0.1712	0.1142	0.0629	0.0215	0.0009
0.8	0.4493	0.3847	0.3216	0.2603	0.2014	0.1454	0.0937	0.0485	0.0143	0.0003
0.9	0.4066	0.3456	0.2864	0.2291	0.1745	0.1232	0.0766	0.0370	0.0093	0.0001
1.0	0.3679	0.3105	0.2549	0.2015	0.1510	0.1041	0.0623	0.0280	0.0060	0.0000

(B) Backward simulation; values of N_{t-1}/N_t against M and C_{t-1}/N_t

	C/N										
M	0	0.3	0.6	0.9	1.2	1.5	1.8	2.1	2.4	2.7	3.0
0.1	1.1052	1.4198	1.7333	2.0461	2.3583	2.6700	2.9814	3.2924	3.6031	3.9135	4.2238
0.2	1.2214	1.5511	1.8787	2.2048	2.5296	2.8535	3.1766	3.4991	3.8209	4.1422	4.4630
0.3	1.3499	1.6951	2.0373	2.3771	2.7151	3.0517	3.3870	3.7213	4.0546	4.3872	4.7191
0.4	1.4918	1.8532	2.2104	2.5645	2.9161	3.2658	3.6138	3.9604	4.3057	4.6500	4.9933
0.5	1.6487	2.0267	2.3995	2.7683	3.1341	3.4973	3.8585	4.2178	4.5756	4.9320	5.2872
0.6	1.8221	2.2172	2.6060	2.9902	3.3706	3.7479	4.1227	4.4953	4.8660	5.2350	5.6025
0.7	2.0138	2.4264	2.8319	3.2318	3.6274	4.0193	4.4082	4.7946	5.1786	5.5607	5.9411
0.8	2.2255	2.6563	3.0789	3.4951	3.9064	4.3134	4.7170	5.1176	5.5155	5.9112	6.3048
0.9	2.4596	2.9090	3.3492	3.7823	4.2097	4.6324	5.0512	5.4665	5.8789	6.2886	6.6959
1.0	2.7183	3.1868	3.6451	4.0956	4.5398	4.9787	5.4131	5.8438	6.2710	6.6953	7.1170

Gastric Evacuation in Walleye Pollock, *Theragra chalcogramma*[1]

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The effects of meal size and temperature on food evacuation in juvenile walleye pollock (*Theragra chalcogramma*) were examined. In the range of meal sizes used (0.5–2.5% of body weight), food evacuation was a negative, exponential function of time. Increasing meal size reduced instantaneous evacuation rate but increased the amount of food evacuated. In the range of temperatures used (3–9°C), both instantaneous evacuation rate and amount of food evacuted were positive linear functions of temperature. Our evacuation rate values are higher than previous estimates for walleye pollock, suggesting that daily ration and the trophic impact on prey species may have been significantly underestimated.

Les effets de l'importance du repas et de la température sur l'évacuation chez la morue du Pacifique occidental juvénile (*Theragra chalcogramma*) ont été examinés. Dans la plage d'importance des repas utilisée (0,5–2,5% du poids), l'évacuation était une fonction exponentielle négative du temps. Le fait d'augmenter l'importance des repas réduisait le taux d'évacuation instantané, mais augmentait la quantité évacuée. Dans la plage des températures utilisées (3–9°C), le taux instantané d'évacuation et la quantité évacuée étaient des fonctions linéraires positives de la température. Les taux d'évacuation obtenus sont supérieurs à ceux d'estimations précédentes chez cette espèce, ce qui peut laisser à penser que la ration quotidienne et l'effet trophique sur les espèces qui servent de proie ont pu être sous-estimés de façon importante.

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The fishery for walleye pollock, *Theragra chalcogramma*, in Alaskan waters fluctuates around 1 million metric tonnes per year. Information about the physiology and biology of walleye pollock is incomplete but essential for an understanding of the ecosystem in which this exploitable stock is found and essential to refine the simulations, such as that of Laevastu and Larkins (1981), now being used to manage them.

Aspects of the morphology and physiology of pollock have been studied by various authors (Hamai et al. 1971; Haryu 1980; Maeda et al. 1981; Paul 1983, 1986; Smith and Paul 1986; Smith et al. 1988) but studies of feeding and digestive physiology are few (Yoshida and Sakurai 1984; Smith et al. 1986; Dwyer et al. 1986; Dwyer et al. 1987).

Instantaneous gastric evacuation rates are commonly used to calculate daily ration. This study examines food evacuation in walleye pollock held at temperatures characteristic of their natural Alaskan environment. In particular, we examine the effects of temperature, meal size, fish size, and multiple meals on food evacuation. We also examine the interrelationship between evacuation and time between meals.

Daily ration information is used in modeling pollock production and in evaluating its ecological impact on prey populations (Laevastu and Larkins 1981; Dwyer et al. 1986; Livingston et al. 1986). To date, much of the data used in the production model has been derived from studies of different species or from pollock studies done at higher temperatures than are applicable to Alaskan conditions. Daily ration estimates derived from the results of this study are compared with previous estimates for pollock, suggesting that pollock impact on their prey may have been underestimated.

Materials and Methods

Fish were captured in Resurrection Bay, Alaska, in shrimp pots or by hook and line and returned to the laboratory at Seward Marine Center immediately after capture. In the laboratory, fish were held in circular seawater aquaria containing about 800 L of seawater. Fish were held in captivity until they fed readily before being used in experiments.

Experiments on the effects of temperature, meal size, and multiple meals on gastric evacuation and on the effect of feeding interval on food intake were made on pollock weighing 30–70 g. Pollock ranging in size from 30–660 g were used in the experiment on the effect of fish size on evacuation.

In the evacuation experiments, each fish was anesthetized (MS-222), weighed, measured for fork lengths, allowed to recover from anesthesia, and fasted for 48h immediately before the start of each experiment. Fish ingested food voluntarily. The food used in the experiment on fish size was whole pink shrimp, *Pandalus borealis*, one of their natural prey. In other experiments, the food was glass shrimp (*Pasiphaea pacifica*)

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TABLE 1. Instantaneous evacuation rates (b) for pollock experiments at three different temperatures (3°, 6°, and 9°C) at three different meal sizes (0.5, 1, and 2.5% of body weight). Linear values were derived from the equation: $W_t = W_0 - bt$. Exponential values were derived from the equation: $W_t = W_0 e^{-bt}$. Both daily (d) and hourly (h) rates are listed. Coefficients of determination (r^2) are listed for all the regressions from which b values were derived.

Temperature (°C)/ meal size (%)	Rate	Linear values: Dry weight	r^2	Ash-free dry wt	r^2
3,1	d	−0.4129	0.91	−0.4080	0.94
	h	−0.0172		−0.0170	
6,0.5	d	−1.529	0.71	−1.503	0.76
	h	−0.0637		−0.0626	
6,1	d	−0.7829	0.91	−0.7938	0.93
	h	−0.0326		−0.0330	
6,2.5	d	−0.4215	0.98	−0.4169	0.99
	h	−0.0176		−0.0174	
9,1	d	−1.033	0.92	−1.008	0.88
	h	−0.0430		−0.0420	
		Exponential values			
3,1	d	−0.8186	0.99	−0.7781	0.98
	h	−0.0341		−0.0324	
6,0.5	d	−2.276	0.88	−2.041	0.91
	h	−0.0948		−0.0850	
6,1	d	−1.460	0.98	−1.377	0.99
	h	−0.0608		−0.0574	
6,2.5	d	−0.8531	0.98	−0.8156	0.97
	h	−0.0355		−0.0340	
9,1	d	−2.250	0.97	−2.242	0.90
	h	−0.0937		−0.0934	

which are similar structurally to euphausiids, the primary crustacean prey of pollock. Glass shrimp are larger than euphausiids, however, making it possible to feed pollock only a single organism to meet the ration requirements and thus eliminate the confounding effect of varying surface area with the number of individual prey items. Three different meal sizes were used: a wet weight of prey equal to 0.5% of the predator's body weight, 1.0% body weight, or 2.5% body weight. In all cases, food consisted of a single whole prey organism. These meal sizes were selected because they are within the range of rations voluntarily ingested in a single feeding in the laboratory (Smith et al. 1986) and are similar to the weights of stomach contents seen in field sampling of pollock (Livingston et al. 1986). In the multiple meal experiment the initial meal size was 2.5% of fish body weight. A second meal, consisting of 1.25% of body weight, was presented after 50% of the initial meal was evacuated as estimated in the single meal experiment.

Three temperatures were used: 3°C ±0.5, 6°C ±0.5, and 9°C ±0.5. The 3°C experiments were conducted in a refrigerated room and the others at the natural temperature of the incoming water at Seward Marine Center. Fish were acclimated to all temperatures for 21 d before experiments and were fed daily.

Sampling times ranged from 6 to 48 h after the meal. From three to seven replicates were obtained at each sampling time. Each fish was sacrificed, stomach removed, and contents placed in a preweighed crucible. Wet weight was calculated and each crucible was placed in a drying oven (60°C) for 48 h. Dry weight was obtained and each crucible was placed in a muffle furnace (500°C) for 12 h. The remaining ash was weighed; these different weights allowed calculation of the percent of the initial meal remaining, in terms of wet weight, dry weight, and ash-free dry weight, at each sampling time. These percent values were used to calculate instantaneous evacuation rates. Both linear ($W_t = W_o - bt$) and exponential ($W_t = W_o e^{-bt}$) equations were employed where W_t is weight remaining at time t; W_o is initial weight; b is instantaneous evacuation rate; and t is time (either in days or hours).

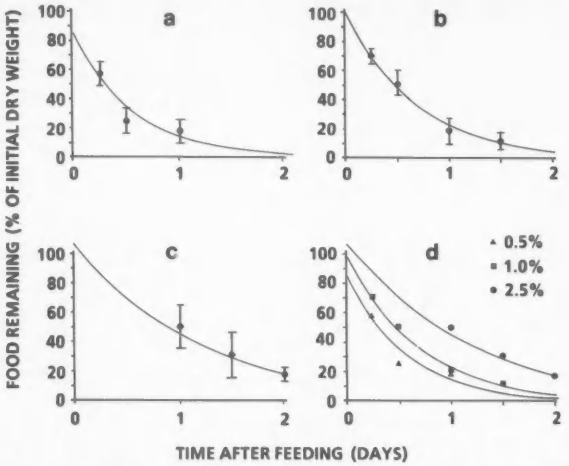

FIG. 1. Effect of meal size on gastric evacuation in 30–70-g walleye pollock. Each meal consisted of a single glass shrimp; experimental temperature was 6°C. (a) meal size = 0.5% of body weight, (b) meal size = 1.0%, (c) meal size = 2.5%, (d) composite of a, b, and c. Data points in a, b, and c are means ± 1 SD.

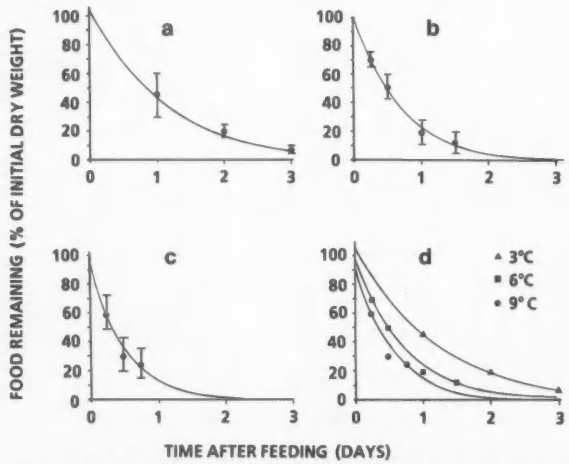

FIG. 2. Effect of temperature on gastric evacuation of a 1% of body weight meal (a single glass shrimp) from the stomachs of 30–70-g walleye pollock. (a) 3°C, (b) 6°C, (c) 9°C, (d) composite of a, b, and c. Data points in a, b, c are means ± 1 SD.

The effect of fish size on evacuation was studied by feeding each fish a meal equal to 1.0% of its body weight. All fish were sacrificed 24 h after the meal, and the stomach contents were handled as above.

An experiment to test the effect of multiple meals on evacuation rate was performed on 30–70-g fish. The experiment

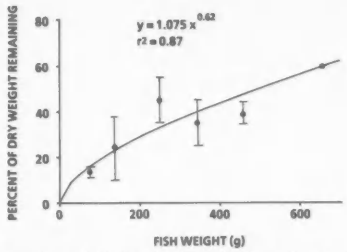

FIG. 3. Evacuation of a 1% of body weight meal, a single pink shrimp, from the stomachs of walleye pollock of 30–660 g body weight. Experimental temperature was 6°C. Vertical bars indicate ± 1 SD.

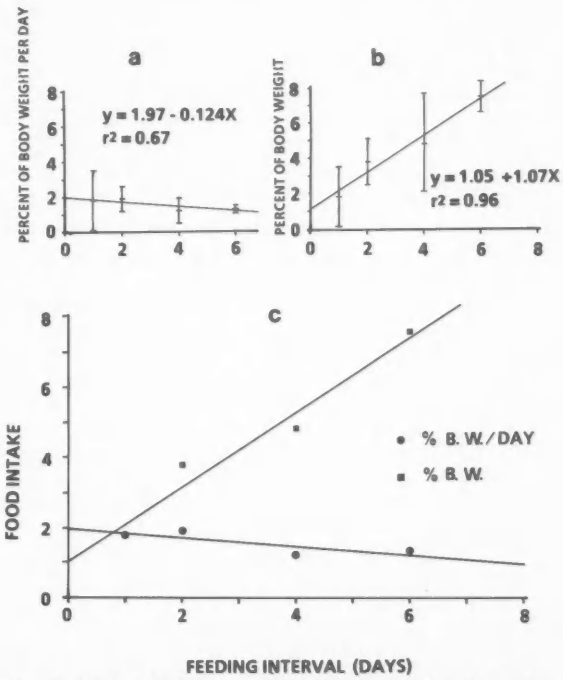

FIG. 4. Effect of feeding interval on food intake in 30–70-g walleye pollock held for 30 d at 6°C. Feeding intervals were every 1, 2, 4, and 6 d. (a) food intake, as a percent of body weight, per day, (b) food intake, as a percent of body weight, per feeding, (c) combined plot of a and b. Data points in a and b are means ± 1 SD.

consisted of five treatment groups of three to five fish each. The groups were maintained in running seawater at 6°C for 30 d; each group was fed to satiation on a different feeding interval: daily, every 2, 4, 6, and 8 d. The amount of food ingested was measured for each feeding (amount available − amount not eaten), allowing calculation of mean food intake per feeding and per interval.

To test for significance of temperature and meal size, the slopes of the linear regressions were compared using a *t*-test statistic described in Zar (1971).

Results

Instantaneous evacuation rates derived from the mean weights of stomach contents remaining at each time interval, fitted to both linear and exponential models, are listed in Table 1. All fishes examined had material in their stomachs at the time of examination. Coefficients of determination (r^2) were calculated for each data set fitted to both linear and exponential models. With one exception, the exponential regressions yielded higher r^2 values than the equivalent linear regressions. The exception was the largest meal size, 2.5% of body weight; the linear equation and the square root model provided an equally good fit of the data.

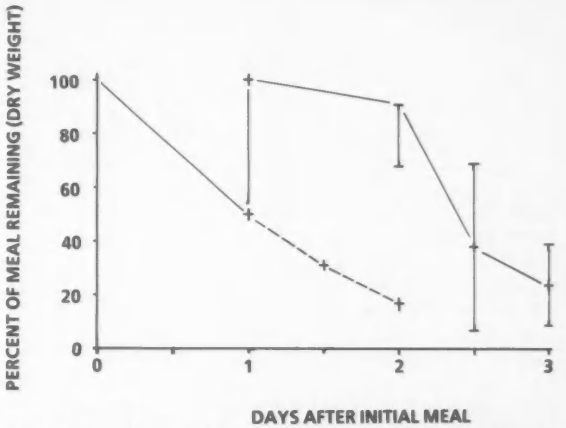

FIG. 5. Effect of a second meal on gastric evacuation in 30–70-g walleye pollock at 6°C. Initial meal was 2.5% of body weight; the second meal (1.25% of body weight) was ingested 24 h after the initial meal and returned the stomach to the initial level of fullness. A single glass shrimp was used as food in each meal. Vertical bars indicate ± 1 SD.

The evacuation rates of the three different meal sizes (Fig. 1) differ significantly from each other ($p \leq 0.01$). These rates decrease with increasing meal size, allowing the calculation of a regression of gastric evacuation time (time to remove 50% of the meal) on meal size that follows the equation $y = 11.4\,x^{0.68}$ ($r^2 = 0.98$). The actual amount of food evacuated per unit time is increased with increasing meal size, conforming to the equation $y = 0.256\,x^{0.58}$ ($r^2 = 0.96$).

Three different test temperatures resulted in significantly different ($p \leq 0.05$) instantaneous evacuation rates (Fig. 2). The amount of food remaining in the stomach 24 h after ingestion of a 1% of body weight meal is a power function of fish body weight (Fig. 3).

The relationship of food intake per feeding interval (% of body weight per day) to feeding interval (Fig. 4a) and of food intake per feeding to feeding interval (Fig. 4b) indicate that extending the interval between feedings results in larger food intake per feeding. Thus, there is little effect on the average food intake over a longer period under the experimental conditions we employed. For example, fish feeding at 6-d intervals had average food intake rates similar to those of fish fed daily (Fig. 4a).

The results of the multiple meal experiment are seen in Fig. 5. The second meal refilled the stomach to a slightly greater extent than initially. The second meal resulted in a lag phase of evacuation that lasted at least 24 h, followed by an extremely rapid evacuation of the entire contents in the second 24 h. The overall effect of the second meal, considering the 4-d period following the initial meal, was to produce an evacuation of food about 10% less than that which would result from two single meals spaced 48 h apart.

Discussion

Most of our results are consistent with exponential models of gastric evacuation. However, the largest meal size employed, 2.5% of body weight, yielded data best fit by the square root model. Previous studies of gadid stomach evacuation produced a variety of results. Jones (1974) reported linear stomach depletion in haddock (*Melanogrammus aeglefinus*) fed meals of 1.8 and 5.4% of body weight. Tyler (1970) described gastric evacuation of juvenile Atlantic cod (*Gadus morhua*) as an exponential function. Daan (1973), also working on Atlantic cod, uses a linear depletion function contending that Tyler's functions were curvilinear only if the last 10% of the meal is considered, an unrealistic assumption for natural populations. Jobling (1981) recalculated the data of Tyler and similar data on Atlantic cod from Bagge (1977) and concluded that the square root model was a better predictor than either exponential or rectilinear models. Based on an extensive review of several species, Persson (1986) concluded that exponential models are generally better predictors than linear or square root.

Elliott and Persson (1978) suggested that the exponential nature of the evacuation relationship is apparently unaffected by meal size. In contrast, Jobling (1986) used computer simulations of gastric evacuation incorporating digestive physiological relationships and predicted that a linear expression would best describe the emptying of large sized particles. In this present study, small prey were evacuated exponentially; the largest prey was evacuated in a linear (square root) fashion. Thus, our results are consistent with Jobling's prediction. Dwyer et al. (1987) also report a curvilinear gastric evacuation in walleye pollock.

Our exponent in the relationship between gastric emptying time (GET) and meal or particle size (0.68) is in good agreement with the theoretical value of 0.67 predicted by Fange and Grove (1979). Thus, our findings support the surface area hypothesis predicting digestion proportional to size $^{(0.67)}$. The relationship between food evacuated and fish body size (0.62) also agrees well with the theoretical expectation.

We demonstrated previously (Smith et al. 1986) that with increasing temperature, food consumption in juvenile pollock significantly increases. In most fishes increased temperature also results in increased gastric evacuation rate, usually following an exponential or power relationship (Elliott and Persson 1978). Elliott (1972) expressed the relationship as

$$R = ae^{bT},$$

where R = evacuation rate, a is a constant relating to prey type, and b is another constant. Durbin et al. (1983) review previous evacuation studies, estimate the value of b as 0.115 and use that b to calculate evacuation rates and daily rations for silver hake (*Merluccius bilinearis*). Dwyer et al. (1986) also use that b value to scale evacuation rates obtained on walleye pollock at 8.5–9.0°C to lower temperatures more representative of Bering Sea conditions. Our data were used to calculate an exponential equation, the b constant of which was 0.168. Instantaneous evacuation rate (R) derived in this study, that estimated by Dwyer et al. (1986), and that extracted from Tyler's (1970) study of cod are 0.034, 0.011, and 0.054, respectively, at 3°C. The mean weight of pollock used in the Dwyer et al. (1986) study was 460 g; the mean weight in this study was 50 g. We used the power function described in Fig. 3 to scale our 3°C rate to a 460-g pollock. The dry weight remaining was used to calculate an evacuation rate for this size fish. The resultant R value of 0.013 is very similar to the Dwyer value. However, our study employed voluntary feeding while Dwyer et al. (1986) used force feeding, a stressful procedure known to affect gastric evacuation in several fish species (Talbot 1985). Therefore, the 0.011 value possibly derives from two compensating factors, a low temperature scaling factor and a low evacuation rate measured at 9°C. While the Dwyer et al. (1986) methodology predicted evacuation rate well for a 460-g pollock at 3°C, it would not do so at other temperatures and fish sizes. For example, our equations predict evacuation rates of -0.061 and -0.022 for 50 and 460-g pollock at 6°C and a meal size of 1% of body weight. Dwyer's methodology predicts rates of -0.018 for both, values 70 and 22% below ours. A bottom temperature of 6°C is one that is typical of the Gulf of Alaska for much of the year (Smith et al. 1988) and is experienced by pollock in the Bering Sea in summer (Chen 1983).

One rationale for measuring evacuation rate is to allow calculation of daily ration. The Elliott and Persson (1978) model has been used by Dwyer et al. (1986) and Dwyer et al. (1987) to calculate daily ration for walleye pollock, but both studies used evacuation rates of about 0.011. Thus the daily ration and the potential ecological impact of this important species may have been underestimated considerably according to the comparison above.

In our multiple meal experiment, a second meal following a large initial meal resulted in a lag phase of gastric emptying (Fig. 5), a phenomenon also reported for Atlantic gadids (Jones 1974), Altantic cod, and several flatfishes (MacDonald et al. 1982). Pollock fed large multiple meals had no apparent advantage in terms of amount of food processed (Fig. 5). This finding led to the question: are gastric evacuation and feeding periodicity linked? Figure 4 suggests that they are. When fed daily, pollock food consumption is variable; on some days the fish do not feed. Offering food on other than daily schedules allowed time for complete gastric evacuation and a return of hunger. Huebner and Langton (1982) reached the same conclusion about winter flounder, *Pseudopleuronectes americanus*. These results suggest that walleye pollock in the natural environment are adapted to either feed at moderate levels on a continuous food supply or to ingest very large meals when food is scarce. Either of these cases would result in similar food consumption up to some critical, as yet undetermined, feeding interval.

These results are the first detailed estimates of gastric evacuation rates of walleye pollock, one of the most abundant fish species in the North Pacific. Our equations relating meal size, temperature, and fish size allow for estimates of prey consumption rates in this species. Future studies on the effect of prey type and size on evacuation rates would compliment the existing information.

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Zinc Avoidance by Fathead Minnows (*Pimephales promelas*): Computerized Tracking and Greater Ecological Relevance

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Male fathead minnows (*Pimephales promelas*) avoided 0.284 mg·L^{-1} of zinc when given a choice of clean and dosed sides of a featureless openfield tank, as frequently used in avoidance/preference experiments. More than six times as much zinc (1.83 mg·L^{-1}) was required to cause avoidance when males were allowed to establish a territory under a shelter on the side of the experimental chamber which was later dosed. Activity increased during zinc dosing when a shelter was present but did not change in no-shelter tests. Control period activity was positively related to strength of avoidance response. By incorporating relevant motivational factors, behaviour observed in avoidance experiments should be more predictive to nature. A new computerized tracking system automatically recorded movements of the fish, calculated various measures of locomotor response and filed results in preparation for statistical analysis.

Des mâles du tête-de-boule (*Pimephales promelas*) évitent le zinc en concentration de 0,284 mg·L^{-1} quand ils ont la possibilité de choisir entre une eau pure et une eau contenant du zinc dans un bassin qui n'a aucune caractéristique particulière, comme on en emploie fréquemment dans les expériences sur les comportements de préférence et d'évitement. Il faut plus de six fois la dose (1,83 mg·L^{-1}) pour susciter un comportement d'évitement lorsqu'on permet aux mâles de délimiter un territoire sous un abri du côté expérimental du bassin dans lequel on verse éventuellement le zinc. L'activité des sujets s'accroît durant la période d'application du zinc quand il y a un abri, mais ne change pas dans les tests sans abri. L'activité durant la période témoin est en relation positive avec l'intensité du comportement d'évitement. Si l'on tient compte des bons facteurs de motivation, le comportement observé dans les expériences sur l'évitement devrait aider à prévoir le comportement de sujets à l'état sauvage. Un nouveau système informatisé de poursuite enregistre automatique le mouvement des poissons, prend différentes mesures des réponses locomotrices et calcule les résultats sous une forme qui se prête à l'analyse statistique.

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Behavioural change is one of the many ways that aquatic animals respond to and deal with stresses imposed by pollutants. Change in behaviour of fishes may occur at pollutant concentrations that are quite different from those causing significant physiological and biochemical effects, and can be the most sensitive and important effect (Geckler et al. 1976).

Locomotor behaviour in the form of avoidance and preference reactions is the most commonly studied of behavioural responses to pollutants, but no standardized methodology yet exists (Atchison et al. 1987). This is largely due to the variety of apparatus and experimental design, high variability in response, and the difficulty of extrapolating results to nature (Kleerekoper 1976; Westlake 1984). Most experiments give test animals a simple binary choice between clean and dosed areas of an experimental chamber. Testing a number of concentrations determines whether the animal has a directed response to a pollutant and if so, at which concentrations avoidance or preference is displayed. This approach may have limited relevance to real environments where the location of an animal is influenced by the interaction of many other motivational factors such as physicochemical conditions, food, shelter, and reproduction (Sprague et al. 1965; Swenson 1978; Laughlin et al. 1978; Ross and Siniff 1980). Laboratory tests should be more predictive to nature if they consider the normal life habits of a species (Olla et al. 1978; Pinto et al. 1984; Lemly and Smith 1985; Scherer and McNicol 1986; Henry et al. 1986).

The objectives of this study were to develop a standard laboratory approach which superimposed avoidance/preference tests on a natural motivational factor, and to quantify the importance of that factor. Fathead minnows (*Pimephales promelas*) were used because they are a standard test species in physiological toxicity tests and are easy to breed in the laboratory. More importantly, breeding males establish territories under overhanging objects, and this seemed useful as the natural motivational element. Zinc was used as a standard toxicant known to elicit avoidance reactions in several species of fish, from both laboratory and field observations (Sprague 1964, 1968; Sprague et al. 1965; Black and Birge 1980; Brown et al. 1982). One series of tests used a featureless experimental chamber. A second series incorporated a shelter favoured by breeding males (McMillan and Smith 1974), with the hypothesis that this would partially override avoidance reactions to zinc. A computerized

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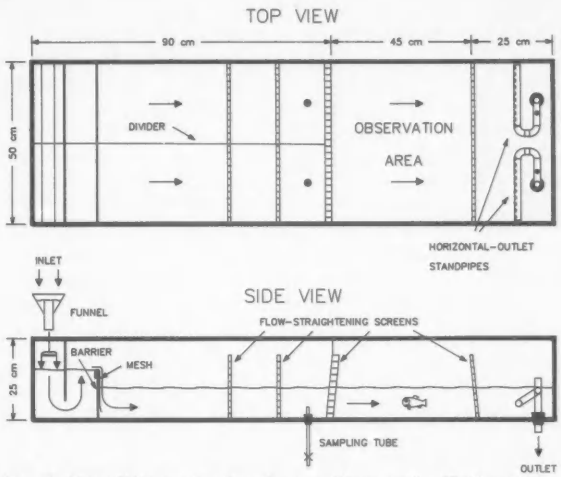

FIG. 1. One of the two laminar flow avoidance tanks. Toxicant from a Marriotte bottle was introduced into a side through either one of two funnels at the upstream end of the channels.

tracking system was developed to standardize observations and to allow prolonged observation periods that would not be feasible with manual tracking by an investigator.

Materials and Methods

Avoidance Apparatus

Two identical avoidance chambers (Fig. 1) were made of clear plexiglass, similar to the design of Westlake and Lubinski (1976). Each observation area was 45 × 50 cm, and it was intended to have laminar flow, with a sharp, vertical interface between the two water-masses moving through. A thin divider formed two channels at the upstream end. Water entered each channel from a funnel, flowed past a baffle, and over a 15-cm high barrier covered by fine Nitex mesh that equalized flow rates. A series of flow-straightening screens, made from "eggcrate" lighting panels covered by 1050-μm Nitex mesh, reduced turbulence and produced essentially laminar flow in the observation area. Screens demarcating the observation area leaned inwards slightly to eliminate dark areas at the periphery of the video camera image. Downstream horizontal outlets maintained water depth at 10 cm. The bottom and sides of the chamber were white; informal tests did not indicate that fish preferred darker backgrounds or behaved differently on them. The chambers rested on a foam bed within a wooden frame covered by black polyethylene, to reduce external mechanical and visual stimuli. Trials with dye solution showed uniform mixing before water reached an observation area, with a relatively sharp and stable interface between the two water masses. Maximum lateral deviation of the interface was 6–7 cm in either direction at the downstream end of the observation area.

The chambers were uniformly illuminated by indirect lighting (250–350 lux at the water surface). Two independently-dimmed rows of four incandescent bulbs were placed just above the upstream and downstream ends of the observation areas. Light from the bulbs, shielded from direct view of the fish and overhead video camera, reflected into the observation area from a white panel 75 cm above the water surface. Portions of the panel were covered with dark paper to minimize glare from the water surface.

Well-water, filtered through nylon aquarium wool in a head tank, flowed through a stainless-steel counter-current heat exchanger to gain heat from used water. From a subsequent tank containing immersion heaters, water was pumped to a final head tank, and then ran down four 1-m long gas-equilibration columns of 3-mm Nitex mesh, which fed the two pairs of channels. Flow rate in each channel was 3.5 $L \cdot min^{-1}$, giving a velocity of 14 $cm \cdot min^{-1}$, and a traverse time of about 3.2 min through an observation area. Float switches in the heating and final head tanks could detect slight drops in water level, prompting a warning and a notation in the experimental record, but this never happened during experimental runs.

A computerized tracking system was developed to monitor locomotion of the fish. It operated in real time and wrote data directly to disk, ready for analysis. The main hardware components were: a low-light black and white video camera with macro zoom lens; a video monitor; and an IBM personal computer with a colour monitor, a printer, and a video digitizing board installed in an expansion slot (PC-Mate Video Van Gogh by Tecmar Inc.). The board could digitize all or part of the video image to a maximum resolution of 256 × 256 pixels. Each pixel could be assigned a "grey level value" corresponding to the brightness of the video image at that location. At the beginning of an experiment, a range of grey values was assigned for the fish, and all values above or below that were designated "not fish," i.e. the observation area.

Software was written in BASIC and Macro Assembler to distinguish an object from a contrasting background and assign x,y coordinates to its location. Various measures of locomotor behaviour were calculated from sequential locations and these could be displayed, printed, and converted to files readable by most statistical packages. The software allowed the object to "disappear" behind an occlusion (the shelter in these experiments), assigning a location at the centre of the occlusion, then resumed normal tracking when the object reappeared. The program also scanned a warning lamp beside the observation area every 15 s to check for low water levels. Programs were menu driven. While designed primarily for behavioural toxicology with fish, the system could, for example, track movements of a small organism on a microscope slide or a larger terrestrial organism. Additional detail is given in Korver (1986) and Korver and Sprague (1988).

Test Fish and Experimental Procedures

Fathead minnows were from laboratory stock of the United States Environmental Protection Agency's laboratory in Duluth, Minnesota. At Guelph, fish were held in well-water at 20°C in 90-L glass aquaria with volume and water-renewal exceeding recommendations of Sprague (1973). Overhead fluorescent Vitalite tubes simulated sunlight with a 16L:8D photoperiod. Daily ad libitum feeding alternated frozen brine shrimp (NUTRAFIN San Francisco Bay Brand; 5.02% minimum crude protein, 0.24% minimum crude fat, 0.29% maximum fibre, 90% maximum moisture) and grower pellets for salmonids (Martins Feed Mills, Martins 84G, 40% minimum crude protein, 10% minimum crude fat, 3.0% maximum crude fibre).

Fish spawned under half-tiles in breeding aquaria, and the next generation (used in experiments) was raised under the same conditions in separate aquaria. Males in breeding condition were selected for experiments when they averaged 4.6 ± 1.0 g (mean

± SD; $n = 50$) in wet weight and 72 ± 5 mm ($n = 50$) in total length. Mortality was negligible throughout.

Two series of tests were performed on experimentally naive fish. In the "no-shelter" series, a featureless observation area was used; in the "shelter" series, a shelter was placed in the middle of the side of the observation area which was to receive the toxicant. The rectangular arch-like shelters were constructed from 3-mm white plexiglass and measured 10 × 10 × 3 cm.

Four concentrations of zinc plus a zero-dose control were tested from the nominal series 0.0056, 0.0393, 0.281, 2.00, and 14.0 mg $Zn \cdot L^{-1}$, representing seven-fold increases. The highest concentration was omitted from no-shelter tests, and the lowest was omitted from the shelter tests. This shortening of the experiment was possible since estimates of avoidance thresholds did not depend on direct statistical comparison of no-shelter and shelter trials. The experiment was run on nearly consecutive weekdays between October 1985 and February 1986, with each of the 10 treatment groups (five test concentrations, with and without shelter) assigned randomly within five replicates blocked in time. Tests on successive days alternated between the two avoidance chambers, and the dosed side of the tank was alternated between each successive replicate in a treatment group.

An acclimation protocol reduced variation in the initial behaviour of fish in the avoidance chambers. This was particularly important for shelter tests, because fish varied in time taken to establish territories underneath the shelter. Fish to be tested were kept for 3 d in a preacclimation tank containing a shelter and two juvenile "companion fish." The companion fish minimized listlessness among test animals kept in prolonged isolation. Lemly and Smith (1985) found a similar protocol necessary. By the third day, all test fish spent nearly all their time under the shelter. Pellet food was used during the preacclimation period but fish were not fed in the avoidance chambers.

Fish were carefully moved from preacclimation to one of the avoidance chambers about 36 h before the beginning of a test. The shelter, if used, was placed in the predesignated side of the observation area before the fish was introduced. The chamber was sealed by a black polyethylene blind and the camera was positioned. Photoperiod was maintained as in the holding facility. Tests began between 10:30 and noon, at least 2 h after the lights came on. The tracking system was usually calibrated the previous evening, and a timer in the program started the test automatically.

The tracking system was set to scan for the fish every half second, centering on its previous location with a movable "scanning window" covering an approximate 14-cm square in the observation area. The instantaneous location of the fish was written to diskette every 15 s, as was the cumulated distance travelled during that period. If the fish swam quickly out of the scanning window or entered the shelter, the whole observation area was scanned every 2.5 s until the fish was again located.

A 3-h control period was followed by 3 h of dosing with zinc solution added to one channel by a Marriotte siphon. A water sample was taken from the middle of the dosed channel just upstream of the observation area (Fig. 1).

Summarized data were printed at the termination of a test to check for anomalies in behaviour. Criteria for rejection of a test were set a priori to allow for normal variation in behaviour of fish while establishing some standardization in potential exposure to the toxicant. No-shelter tests were rejected if, during the 3-h control period, a fish swam less than 36 m and fewer than 5% of the observations at 15-s intervals were recorded on either side of the tank. This was based on preliminary trials which showed an occasional fish remaining motionless on one or the other side of the chamber for very long periods of time during the control period. The criterion ensured that fish used in statistical analysis were at least moderately active and would have some opportunity to discriminate between water-masses in the observation area. For the shelter series, trials showed that most fish had a strong preference for the shelter, and a test was rejected if fewer than 25% of the observations were recorded under the shelter during the control period. Only five of the no-shelter tests, and one of the shelter tests were rejected. These were redone in 2 or 3 d as the acclimation protocol allowed.

Zinc Concentrations and Water Characteristics

Stock solutions of zinc were prepared daily using reagent grade $ZnSO_4 \cdot 7H_2O$ in deionized water acidified to pH <3. Water samples were stored in linear polyethylene bottles which had been bathed in 50% v/v HNO_3 for 24 h and rinsed five times with double deionized water. One millilitre of "Ultrex" ultrapure HNO_3 per 100 mL of sample was used for preservation.

Geometric means of measured zinc concentrations were used in analysis of results. These were 0.020 (zero-dose control), 0.027, 0.060, 0.284, 1.83, and 13.5 mg $\cdot L^{-1}$, measured by atomic absorption spectrophotometry with flame volatilization. Samples for controls and the lowest test concentration were near the detection limit, with coefficients of variation (CV) at 16.7 and 6.4%, respectively. Three random water samples analyzed in triplicate had a mean CV of 8.4%. When corrected for background zinc, the mean for the lowest concentration (0.027 mg $\cdot L^{-1}$) was 136% of nominal. Means from the four highest concentrations had a maximum CV of 4.7%, with measured values ranging from 93 to 101% of nominal. Zinc measurements from the no-shelter and shelter series were not significantly different ($P > 0.05$), and were pooled.

The laboratory well-water maintained stable characteristics during the experiments. Temperature in the avoidance tanks was 20.0 ± 0.1°C (mean ± SD; $n = 50$); pH was 8.06 ± 0.04 ($n = 50$); hardness by EDTA titration (APHA et al. 1985) was 318 ± 8 mg $CaCO_3 \cdot L^{-1}$ ($n = 21$); alkalinity to pH 4.5 (inflection point of titration curve; APHA et al. 1985) was 222 ± 2 mg $CaCO_3 \cdot L^{-1}$ ($n = 21$); dissolved oxygen as measured by a YSI Model 51A Oxygen Meter was 8.2 ± 0.2 mg $\cdot L^{-1}$ ($n = 21$) and always exceeded 85% saturation (91 ± 2%; $n = 21$). Conditions in the holding facilities were similar. Other characteristics of the water are given by Dixon and Sprague (1981). The addition of zinc to the well-water depressed pH significantly ($P < 0.05$) in the three highest test concentrations, but not by more than 0.24 units in the highest concentration, and 0.05 units in the remainder.

Statistical Analysis

Of the statistics calculated by the tracking system from raw data (15-s instantaneous observations on location and cumulated distance travelled), preliminary analysis indicated that five were the most pertinent for further consideration: (1) proportion of observations on the dosed side of the avoidance tank; (2) mean time (seconds) spent per trip in the dosed side; (3) mean time spent per trip in the clean side; (4) number of trips to the

dosed side (= number of trips to clean side ± 1); and (5) mean velocity (centimetres per second).

Transformations were necessary for most statistics to meet the assumptions required for analysis. These were applied separately to the values obtained for each fish during the control period and dosing period. Proportion of observations was transformed using the simple arcsine transformation ($Y = \sin^{-1} X^{0.5}$). Mean trip time to either side and mean swimming velocity were log-transformed ($Y = \log (X+c)$, where $c = 1$ for mean trip time and $c = 0$ for mean velocity). Number of trips required no transformation.

Each fish acted as its own control; responses to be analyzed were calculated by subtracting transformed control-period values from dosing-period values. Use of control-period values as covariates was not possible as the assumption of equal slopes between treatments was not upheld. For each of the five measures of locomotor behaviour, one-way analysis of variance (ANOVA) of the resultant values was used to test for change in response between concentrations of zinc. Further details on selection of transformations and methods of analysis are found in Korver (1986).

A few obvious outliers were evident upon inspection of residuals and normal probability plots. These outlying values were replaced by the original mean value for the concentration level at which they occurred. No more than one outlier was replaced for any given statistic.

A slightly modified version of Williams' (1971) test was used to establish lowest observed effect-concentrations (LOEC; Rand and Petrocelli 1985) for each locomotor statistic. Zero was used instead of the zero-dose control mean in the calculation of Williams' *t*-statistic. This provided a more standardized estimate of LOECs for each of the statistics and made their determination insensitive to variation of zero-dose control means about zero. No zero-dose control means were significantly different from zero ($P > 0.05$) using confidence intervals based on appropriately adjusted mean square errors from ANOVA.

Lethality Test

The 96-h LC50 was estimated as a descriptive benchmark, to allow comparison of the avoidance levels and other work. Accepted procedures were used (Parrish 1985), and general conditions were the same as for the avoidance experiment. Three-month-old fathead minnows weighing 0.082 ± 0.037 g ($n = 20$) were used, with 10 fish randomly allocated to six, 2-L cylindrical plastic test containers. Test solutions were renewed daily. Geometric mean measured concentrations of fresh and day-old solutions of zinc were used to estimate the LC50. Measured concentrations after 24 h dropped to an average of 77% of initial values in the three lowest test concentrations, while the two highest concentrations dropped to 66 and 42% of initial values. A suspended precipitate appeared in the two highest concentrations, but samples passed through a saturated 0.45-μm Millipore filter before acidification showed an average of 90% zinc in the dissolved form, unrelated to concentration. Temperature was 19.7 ± 0.3°C ($n = 5$), hardness 335 ± 13 mg $CaCO_3 \cdot L^{-1}$ ($n = 3$), and dissolved oxygen was initially 8.6 mg$\cdot L^{-1}$, and 7.2 ± 0.4 mg$\cdot L^{-1}$ ($n = 6$) after 24 h. Initial pH was 7.91 in control water, and 7.70 in the highest concentration. After 24 h, pH was generally higher but always within 0.30 units of initial values.

Dead fish were removed at geometric time-intervals. Only one mortality occurred after 36 h, indicating that a threshold of acute lethality had been attained. There was no control mortality. Probit analysis by maximum likelihood with weighted least squares (Hubert 1984) estimated the 96-h LC50 as 15.4 mg Zn$\cdot L^{-1}$ with 95% confidence limits of 13.8 and 17.1 mg$\cdot L^{-1}$.

Results

Control-Period Behaviour

Without a shelter, fish did not prefer either side of the observation area. The confidence interval (CI) for proportion of observations on the side to be dosed covered 0.5. Similarly, the CIs for mean trip times to either side showed a wide overlap (Table 1).

The presence of a shelter invoked a strong preference response. Nearly 99% of control-period observations recorded the fish on the side containing the shelter; 92% of the observations found the fish under the shelter (95% CI = 85.7%, 96.2%). Statistics for mean trip time also showed an overwhelming preference for the shelter side during the control period. Control-period activity levels were much lower when a shelter was present. Number of trips to the side to be dosed and mean swimming velocity were only 25% of the no-shelter levels.

Variation in response of fish during the control period was greater in the no-shelter tests than in the shelter ones. Standard

TABLE 1. Summary of control-period locomotor behaviour for fathead minnows in the avoidance chamber ($n = 25$). All statistics were calculated from transformed values, and then converted back to their original scales. CI = confidence interval.

	No Shelter		Shelter	
	Mean	95% CI	Mean	95% CI
Proportion of observations on side to be dosed	0.410	(0.300, 0.525)	0.986	(0.966, 0.997)
Mean time per trip to dosed side (s)	120	(84.8, 168)	2670	(1450, 4910)
Mean time per trip to clean side (s)	178	(119, 266)	7.9	(3.2, 17)
Number of trips to dosed side	36.9	(26.9, 46.9)	9.4	(4.4, 14.4)
Mean velocity (cm$\cdot s^{-1}$)	1.42	(1.04, 1.94)	0.355	(0.192, 0.657)

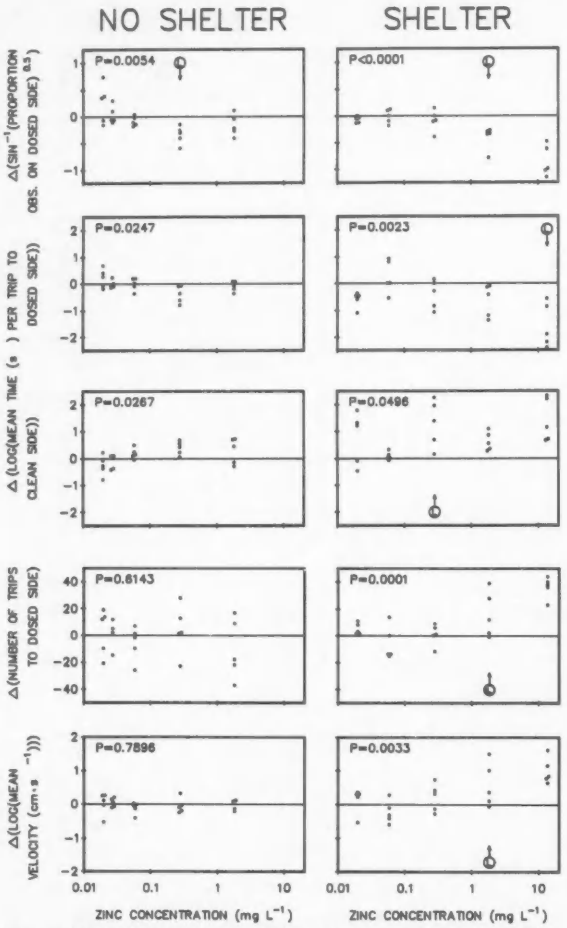

FIG. 2. Effects of zinc concentration on locomotor responses of fathead minnows. Each point on a graph represents the difference between transformed dosing- and control-period values for one fish. *P*-values from analysis of variance are in the upper lefthand corner of each graph. The lowest observed effect-concentration (LOEC) for each statistic is indicated by a circled L.

deviations for no-shelter tests were about twice those of shelter tests for proportion of observations and number of trips. On the other hand, standard deviations for mean trip times and mean velocity were only about half as large in the no-shelter tests ($P < 0.05$; *F*-tests for equality of variance).

Effects of Zinc

A higher concentration of zinc was required to elicit avoidance when a shelter was present, judging by the proportions of observations recorded on the dosed side. The lowest observed effect-concentration (LOEC) for the no-shelter series was 0.284 mg $Zn \cdot L^{-1}$, while that for the shelter series was 1.83 mg $Zn \cdot L^{-1}$ (Fig. 2).

Results were less conclusive for effect of zinc on mean trip time. Analysis of variance found significant differences between zinc concentrations for no-shelter tests, but none of the changes from control-period values were different from zero. With a shelter present, LOECs for mean trip time to the dosed and

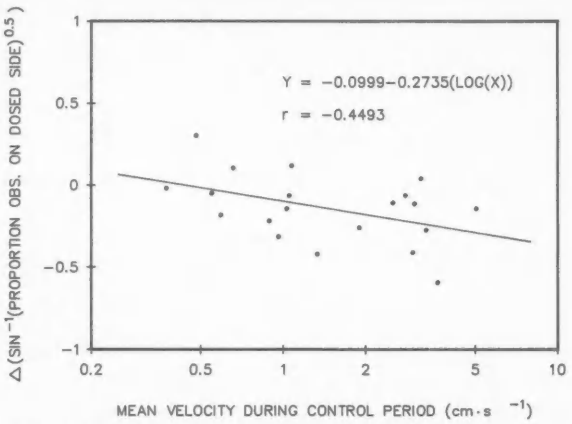

FIG. 3. Relationship between level of activity during the control period and strength of avoidance response for fathead minnows tested with no shelter present. Fish from all test concentrations (excluding zero-dose controls) are included ($n = 20$).

clean sides were 13.5 and 0.284 mg $Zn \cdot L^{-1}$, respectively. The sensitive response for trips to the clean side must be qualified as the ANOVA was marginally significant ($P = 0.0496$) and the small variation for the lowest test concentration resulted in significant heteroscedasticity ($P = 0.0172$; Bartlett's test). Also, at 0.284 mg $Zn \cdot L^{-1}$, one fish was very inactive resulting in an outlier at about -2.4 which was replaced by the original mean response for that concentration. The next highest test concentration (1.83 mg $Zn \cdot L^{-1}$) was accordingly taken as a better estimate of the LOEC.

Fish did not simply move into the clean side when avoiding zinc with a shelter present. They periodically shuttled between the shelter and the clean side, resulting in greater activity relative to the control period (number of trips, mean swimming velocity; Fig. 2). Swimming velocity measured this response with lower variance ($P < 0.05$; *F* based on mean square errors from ANOVA). Activity did not change when there was no shelter, regardless of concentration.

In addition to having higher avoidance thresholds, fish in shelter tests were less effective in avoiding a given zinc concentration. For example, at 1.83 mg $Zn \cdot L^{-1}$ with no shelter, the mean proportion of observations on the dosed side was 0.224. With a shelter, the mean proportion was significantly higher at 0.700 ($P = 0.0127$; two-sample test with means converted back from their transformed values). Even at 13.5 mg $Zn \cdot L^{-1}$ (with a shelter) the mean proportion was 0.353. Absolute mean trip time to the dosed side at 1.83 mg $Zn \cdot L^{-1}$ also showed a significant retained preference for the shelter. Mean values for these statistics were 100 s in no-shelter tests and 268 s with a shelter ($P = 0.0131$; two-sample test). For trips to the clean side the values were 413 and 114 s, respectively, although the difference between these was marginal ($P = 0.0581$).

The more active fish showed stronger avoidance responses in the no-shelter situation. A simple linear regression between relative proportion of observations and mean velocity demonstrated this for all concentrations of zinc, excluding zero-dose controls (Fig. 3). Correlation improved from the indicated value of -0.45 – -0.66 when only the 10 fish from test concentrations at and above the avoidance threshold (0.284 mg $Zn \cdot L^{-1}$) were used.

TABLE 2. Estimates of lowest observed effect-concentration (LOEC) that would be estimated by shortened experiments. The table summarizes analyses of variance and determinations of LOEC for proportions of observations on the dosed side of the observation area, when results were reanalyzed for shortened dosing periods. Control periods were the same duration as the dosing periods, and used the final portion of data records before dosing began. MSE = mean standard error.

	No Shelter			Shelter		
Duration (min.)	MSE	$P(F>F_c)$	LOEC (mg $Zn \cdot L^{-1}$)	MSE	$P(F>F_c)$	LOEC (mg $Zn \cdot L^{-1}$)
15	0.1208	0.0189	1.83	0.0401	0.0002	1.83
30	0.1461	0.1433	>1.83	0.0559	0.0002	1.83
60	0.0304	0.0150	0.284	0.0206	<0.0001	1.83
180	0.0483	0.0054	0.284	0.0371	<0.0001	1.83

Test-periods shorter than 3 h would have given similar estimates of avoidance thresholds, but there were differences between no-shelter and shelter tests (Table 2). Generally, a longer test had less variability and higher levels of significance. With no shelter, mean square error varied by a factor of five over the range of test durations that were compared. For shelter tests, error varied by less than a factor of three and was always less than that for no-shelter tests of the same duration. The LOEC for no-shelter tests did not stabilize to the 3-h estimate until test duration reached 1 h, and in fact the ANOVA for the 30-min dosing period was not significant. With a shelter, the LOEC was constant for all test durations and the significance level was higher, even for the 15-min dosing period, than for the 3-h dosing period with no shelter.

Discussion

When placed in a situation of motivational conflict caused by the presence of a shelter, avoidance thresholds increased by 6.4 times to 1.83 mg $Zn \cdot L^{-1}$. Even at concentrations that elicited avoidance, fish still spent considerable time under the shelter, and tended to shuttle between there and clean water. This would explain the increase in activity over control levels, something that did not happen in no-shelter tests.

The higher avoidance threshold in the presence of a territorial shelter is probably more predictive of what would happen in real waterbodies, where fish have territorial, feeding, and other motivations. Few studies directly compare locomotor responses between laboratory and field because it is difficult to document fish movements in a waterbody and relate them to quantified levels of a pollutant. Nevertheless, motivational factors may explain some discrepancies between laboratory and field observations. Juvenile Atlantic salmon (*Salmo salar*) showed a laboratory avoidance threshold at 0.021 of the lethal concentration of a zinc-copper mixture (Sprague 1964), similar to our no-shelter threshold, 0.018 of the LC50. Adult salmon on a spawning migration did not show avoidance until the metal concentrations were about 18 times greater, in the range 0.35 – 0.43 of the LC50 for juvenile fish (Saunders and Sprague 1967). The authors attributed this large apparent difference to strong migratory motivation. The concentration of another metal mixture (Cu, Cr, As, Se) had to be only twice as high to cause avoidance by fathead minnows in portable enclosures in a natural stream, compared with an artificial stream in the laboratory (Hartwell et al. 1987). In light of our shelter/no-shelter ratio of 6.4, the portable enclosures may not have stimulated appreciable natural motivation to stay on one side or the other. Two related papers on avoidance behaviour of fish to chlorinated discharges (Cherry et al. 1977; Giattina et al. 1981) reported reasonable agreement between laboratory and field thresholds, but only when other factors such as temperature were considered. In another example, laboratory temperature preference of yellow perch (*Perca flavescens*) did not predict response of overwintering fish to a heated discharge; the requirement for overhead ice-cover appeared to override attraction to warm water (Ross and Siniff 1980). Similar discrepancies have been found for invertebrates. Blue crabs (*Callinectes sapidus*) strongly avoided pH 6.0 and lower in the laboratory. Large crabs avoided acidic areas of an estuary in similar fashion, but small ones did not. The investigators (Laughlin et al. 1978) suggested that various factors including migratory and feeding motivation might account for the difference.

All these studies demonstrate, to some extent, the limitations of single-factor laboratory choice tests in predicting avoidance reactions of fish in polluted waterbodies. Nevertheless, single-factor tests may be useful for establishing minimum avoidance thresholds for situations when fish are not strongly motivated to remain in one location. Fathead minnows, for example, are a nonterritorial, schooling species for much of the year (McMillan and Smith 1974), at which time field avoidance thresholds might be close to results obtained for laboratory tests without a shelter. During the summer, male fathead minnows become territorial and spawning occurs repeatedly beneath overhanging objects. Avoidance thresholds may then be maximal, considering the strong retained preference for the shelter during our dosing period. Our laboratory study could thus allow better extrapolation to field situations inasmuch as it defines some scope for avoidance thresholds in fish having different motivational states. Olla et al. (1978) put forward similar arguments to explain the behaviour of the tautog (*Tautoga onitis*) at high, sublethal temperatures.

One important factor that might limit application of results from laboratory studies to nature is the steepness of the toxicant gradient. Gradients in the field tend to be gradual, but most laboratory tests (including ours) have used sharp gradients. Kleerekoper (1973) noted attraction to and avoidance of similar concentrations of copper using shallow and steep gradients, respectively. Some visual observations made during our shelter tests may be relevant to Kleerekoper's findings. At zinc concentrations eliciting avoidance, fish initially responded with agitated movements under the shelter. Then progressively longer forays were made from the shelter, until the toxicant interface was encountered. There, the fish halted abruptly and returned to the shelter. This behaviour was often repeated several times until the fish swam through the interface into clean water. Return trips to the shelter were continued, but having

overcome the "surprise" of the interface, fish spent more time in clean water. In relation to Kleerekoper's observations, fish might have to become accustomed to a steep gradient, regardless of its direction, before being able to assess the relative merits of being on one or the other side of it.

The foregoing discussion largely assumes that results for shelter tests can be explained in terms of motivational conflict. Another possible explanation is evident from the results themselves. As there was a relationship between control-period swimming velocity and strength of subsequent avoidance (Fig. 3), one might hypothesize that differences between avoidance thresholds for the no-shelter and shelter situations were due to differences in activity only. Fish spending most of their time in one location may not be as aware of a choice between polluted and unpolluted areas. Some authors (e.g. Sprague 1968; Stevens et al. 1980) have commented on this as a way of explaining interspecies differences in avoidance responses (i.e. territorial or sedentary species may have higher avoidance thresholds for a given pollutant than nonterritorial or motile species). This might also apply to conspecifics in different motivational states, as shown here. However, the observed relationship between activity and strength of avoidance might just be an artifact of the test chamber. Active fish may disturb the toxicant interface more frequently, and thus move further into the clean side of the tank to avoid laterally dispersed plumes of toxicant.

The no-shelter avoidance threshold of zinc for fathead minnows (0.284 mg $Zn \cdot L^{-1}$) is of intermediate sensitivity compared with other reported values. Sprague (1964, 1968); Black and Birge (1980) and Brown et al. (1982) give lower or much lower thresholds of zinc for salmonids, but Summerfelt and Lewis (1967) found no avoidance by a centrarchid, even at 80 mg $Zn \cdot L^{-1}$. Fathead minnows might show a lower threshold in water of different quality; our well-water was very hard and contained a fairly high concentration of background zinc (0.020 mg $Zn \cdot L^{-1}$). Most metals are physiologically much more potent in soft water, and recent work by Hartwell et al. (1987) suggests that avoidance responses are similarly affected.

Comparison of Avoidance Thresholds to Physiological Endpoints

Our 96-h LC50 of 15.4 mg $Zn \cdot L^{-1}$ at hardness 318 mg $CaCO_3 \cdot L^{-1}$ was within the 95% confidence interval of the value predicted by Spear (1981) from a compilation of cypriniform data. Sublethal effects of toxicants on fish are often expressed as a fraction of the lethal concentration, and such ratios are often in the vicinity of 0.1 LC50, 0.05, or sometimes as low as 0.01. Our avoidance LOECs were in the same general range, being 0.12 with a shelter present, and 0.018 with no shelter.

Apparently, fathead minnows can detect and avoid zinc down to concentrations which cause sublethal physiological effects. The physiological LOEC in a life-cycle test with fathead minnows in hard water was 0.020 of the LC50 (Brungs 1969). Recent results from our laboratory show decreases in nucleic acids of newly-hatched fathead minnows (i.e. growth effects) at 0.013 LC50 (J. Parrott, pers. comm.). These two findings are similar to our ratio of 0.018 with no shelter. In soft water the life-cycle LOEC was 0.24 LC50 (Benoit and Holcombe 1978), less sensitive than the avoidance thresholds found here.

A classic study by Geckler et al. (1976) demonstrated the importance of interactions between behavioural and physiological responses to pollutants in both laboratory and field situations. Copper concentrations which inhibited laboratory spawning by green and longear sunfish (*Lepomis cyanellus* and *L. megalotis*) were considerably higher than these which affected spawning of fish moving freely in a dosed section of experimental stream isolated by barriers. Fish spawned only in low copper concentrations near the downstream barrier, apparently avoiding high concentrations. Similar laboratory and field results were obtained for bluntnose minnows (*Pimephales notatus*), and although the authors did not attribute the results to behaviour of the fishes, an avoidance response provides an appropriate explanation (Atchison et al. 1987).

Experimental Techniques

The daily 6-h test runs used in this work consumed several months. A postdosing control period lengthened our runs to 9 h, but results did not add anything to analyses, and have not been used. Faster tests would make it easier to run more replicates than the bare minimum of five that we used. While long-term behavioural observations are important, 2-h runs, divided equally into control and dosing periods, would apparently provide satisfactory avoidance information (Table 2). Three or four fish could be tested sequentially during a day, given enough replicate test chambers for acclimation. Testing several fish simultaneously would require not only more chambers, but additional cameras and computers because of speed limitations in tracking hardware. The acclimation period might be shortened by having many chambers, used for preacclimation as well as testing, and by removing companion fish instead of moving test fish. Time of acclimation might be reduced by positively conditioning fish to handling, by briefly netting them prior to normal feeding in the rearing tanks (Schreck 1981). Controlling temperature of large amounts of water could also be a factor; we had to build a counter-current heat exchanger which cost more than the computer. The need for replication would be less for shelter tests because estimates of avoidance thresholds were more precise. The greater precision resulted largely from more consistent behaviour during the control period, and from the fish encountering the toxicant at an early stage of the dosing period.

Performance of the Automated Tracking System

The tracking system was generally quite dependable. Once calibrated, it tracked a fish for 9 h with infrequent problems. It sampled location more frequently than a human could do, without fatigue or biased expectation. A truly major saving of time was the automatic and almost immediate tabulation of data into a form ready for analysis, replacing tedious transcription which can take several times as long as the experiment.

The advantages of automated behavioural monitoring of aquatic organisms is reflected by the variety of systems described in the literature (see Gruber and Diamond 1988). Recently, commercially available monitoring systems have become more prevalent, although they are expensive. The system developed here features: (1) inexpensive, PC-based, off the shelf technology; (2) real-time processing with results immediately available for viewing and analysis; and (3) adaptability to a variety of experimental designs.

Nevertheless, some aspects of the system were less than perfect and these were chiefly associated with slow speed of operation and contrast of the fish with its background. Fish sometimes move in rapid bursts and this necessitated algorithms in the software to find the fish if it moved out of the scanning

window. This resulted in brief interruptions of the regular schedule of observations, repressing about 1% of the overall records. The main problem with contrast was small areas of relative darkness at the edges and corners of the test chamber. The tracking system could "lock on" to these areas, but this was very infrequent.

The tracking system likely introduced some bias into results for the shelter tests, especially in measures based on distance travelled. When the tracking system could not find the fish anywhere in the chamber, the coordinates for the centre of the shelter were used as its location (actual algorithms were slightly more sophisticated). Hence, activity under the shelter was largely ignored. No doubt this explains some of the differences between no-shelter and shelter tests for mean swimming velocity. The use of responses based on changes from control-period activity should have eliminated most of this bias within each category of test.

Statistical Considerations

Judging from sensitivity of the avoidance thresholds, the half-second scanning rate with 15-s instantaneous observations written to disk were adequate to estimate time spent on a side. Such observations are not, however, very efficient at assessing sequences, frequencies, or durations of distinct behaviours (Slater 1978). We might have reduced the variability in number of trips, and hence mean trip time, by setting the program to record locations more frequently.

Mean trip time has several advantages as a statistic for measuring avoidance behaviour (de March and Scherer 1980). Movement in either side of the chamber is separated into two potentially independent responses, and differences in activity levels between fish (Fig. 3) are taken into account. However, we found LOECs for mean trip time to be less sensitive than those for time spent on a side (proportion of observations). No LOECs were found for trip time in no-shelter tests. Differences in experimental and analytical technique from those of de Marche and Scherer, and the 15-s sampling interval, likely account for this lack of sensitivity. A better way to integrate time spent and activity might be to calculate total time spent on a side per unit distance travelled on that side. The advantages of a mean time statistic would be retained and error should be less than for mean trip time, since distance travelled was measured more precisely than number of trips.

The determination of LOECs by analysis of variance and the described modification of Williams' (1972) test involved some compromise. We used Williams' test because it has greater power (Gelber et al. 1985) than Dunnett's (1964) more popular procedure. It assumes that toxicant effects increase monotonically with concentration, not unreasonable judging from overall trends in Fig. 2. Unfortunately, the use of control-period behaviour in addition to the zero-dose control required by Williams' test caused some problems. Williams' test looks for significant changes in mean response at the same overall significance level as ANOVA. Although no zero-dose means were significantly different from zero, slight deviations gave LOECs that were higher or lower, depending on the direction of the deviation. Replacing the zero-dose mean in Williams' t-statistic with zero eliminated this problem but led to some inconclusive results. For example, no LOECs were found for mean trip times without a shelter, even though ANOVA found significant differences. The main reason for this discrepancy was probably not so much a change in the statistical power of the modified test, but a change of hypothesis; the standard test finds differences between mean levels at various test concentrations while the modified test looks for differences of mean levels from zero. A recently proposed test (Tukey et al. 1985) may provide an alternative. It is based on modified regression analysis for a significant trend in response with concentration and takes advantage of monotonicity, but does not assume it.

A major limitation of analysis was the lack of consideration of changes in behaviour during the 3-h experimental periods. Data provided by the tracking system are amenable to more sophisticated approaches. Time-series analysis has been employed successfully by some authors in modelling fish behaviour (Matis et al. 1974a, 1974b, 1975; Thompson et al. 1982). Multivariate ANOVA (Srivastava and Carter 1983) and growth models (Carter and Hubert 1984a, 1984b, 1985) should also be explored.

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Prediction Limits of Allometric Equations: A Reanalysis of Ryder's Morphoedaphic Index

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Schneider, D. C., and R. L. Haedrich. 1989. Prediction limits of allometric equations: a reanalysis of Ryder's morphoedaphic index. Can. J. Fish. Aquat. Sci. 46: 503–508.

Empirical equations are frequently used to predict potential fish harvest from lakes or reservoirs. The prediction limits around these estimates have not been evaluated. We found that prediction limits from Ryder's morphoedaphic equation were inaccurate because of artificial correlation between dependent and independent variables used in regression. Furthermore there are inconsistencies between different versions of the model. Reanalysis of Ryder's data, gave upper 95% prediction limits that were 4.8–5.9 times the lower 95% prediction limit. Separating the component variables of the morphoedaphic index reduced prediction limits slightly. New upper limits were 4.5–5.6 times the lower 95% limits. Theoretical scaling of fish harvest on lake volume, consistent with the known tendency of small lakes to yield more fish per unit area, reduced the upper prediction limits to 2.8 times the lower limit. The model, which was allometric in form, correctly related the exponent relating harvest to lake volume in two independent data sets.

On a fréquemment recours à des équations empiriques pour prédire la récolte potentielle de poissons dans des lacs ou des réservoirs. Les limites des prévisions obtenues n'ont pas été évaluées. Nous avons trouvé que celles de l'équation morphoédaphique de Ryder sont inexactes. Cela est attribuable à une corrélation artificielle entre les variables dépendantes et les variables indépendantes utilisées dans la régression. En outre, il existe des inconsistances entre les différentes versions du modèle. Une nouvelle analyse des données de Ryder a donné de nouvelles limites supérieures de prévision de 95% qui étaient 4,8 à 5,9 fois la limite inférieure de prévision de 95%. La décomposition selon les variables de l'indice morphoédaphique a permis de réduire légèrement les limites de prévision. Les nouvelles limites supérieures étaient 4,5 à 5,6 fois les limites inférieures de 95%. Une projection théorique de la récolte de poissons sur le volume des lacs et qui est conforme à la tendance connue des petits lacs à produire davantage de poissons par unité de surface, a permis de réduire les limites supérieures de prévision à 2,8 fois la limite inférieure. Le modèle, de forme allométrique, a prédit correctement l'exposant qui relie la récolte au volume de lac dans deux séries indépendantes de données.

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Allometric equations, which use fractional exponents to relate a dependent variable to one or more independent variables, have become increasingly prominent in ecology (Calder 1984; Peters 1983). Least squares regression is widely used to estimate these fractional exponents, yet little attention has been paid to the uncertainty attending predictions made from equations whose parameters have been estimated statistically. We investigated, and then attempted to reduce, the prediction limits of a widely used allometric equation developed by Ryder (1965) to estimate the potential harvest of fish from lakes and reservoirs.

Ryder (1965) used an exponential equation to describe annual fish harvest per unit of lake area as a function of the morphoedaphic index, which is the ratio of total dissolved solids to mean depth. The form of the equation was:

$$(1) \quad H = c\,\mathrm{MEI}^{b}$$

The variable H is annual fish harvest ($\mathrm{lb \cdot acre^{-1} \cdot yr^{-1}}$), MEI is the morphoedaphic index ($\mathrm{ppm \cdot ft^{-1}}$). Parameter c is a transfer coefficient with units of $\mathrm{lb \cdot acre^{-1} \cdot yr^{-1} \cdot ppm^{-b} \cdot ft^{b}}$. Parameter b is a dimensionless scaling coefficient that describes an allometric relationship between fish harvest and the morphoedaphic index.

Ryder (1965) used data from 23 north-temperate lakes to obtain least squares regression estimates of parameters b and c. Parameter b is the slope of the regression of the logarithm of fish harvest against the logarithm of the morphoedaphic index. Parameter b is calculated from the regression estimate of the intercept. The resultant equation is:

$$(2) \quad H = 2.094\,\mathrm{MEI}^{0.4461}.$$

Ryder's equation has been used frequently to estimate potential fish harvest from lakes and reservoirs where detailed trophic information is lacking. The reliability of estimates from this equation cannot be evaluated because error mean squares of regression estimates were not reported either in the original publication or in subsequent reviews (Ryder et al. 1974; Ryder 1982). We used data reported by Ryder (1965) to estimate the prediction limits of morphoedaphic estimates of fish harvest.

Prediction limits from allometric equations will underestimate true limits if artificial correlation (Calder 1984; Riggs 1962) is present to inflate the explained variance. In the case of the morphoedaphic index artificial correlation arises because area is used to calculate both mean depth (which contributes to variation in the independent variable MEI) and harvest, which

is the dependent variable (see also Youngs and Heimbuch 1982). The artificial correlation can be removed by using the measurement variable (Q = kg·lake^{-1}·yr^{-1}) instead of the derived or standardized variable (H = kg·ha^{-1}·yr^{-1}). Equation 1 then becomes:

(3) $Q = e^g A^h \mathrm{MEI}^i$,

A is surface area (ha),
e^g is an arbitrary constant (kg·yr^{-1}·ha^{-h}·ppm^{-i}·m^i).

Prediction limits, free of artificial correlation, can be estimated by multiple regression.

Prediction limits for allometric equations estimated by regression can be wide because small errors in an exponent have large effects on predictions. We investigated two means of narrowing prediction limits around morphoedaphic projections of fish yield. The first approach was to reduce the level of unexplained variance by separating the morphoedaphic index into its component variables. This approach can reduce the level of unexplained variance if the allometric relation of each of the component variables differs from the exponent in equation 2. To test this we separated variables:

(4) $Q = e^g A^h T^j z^k$

T = total dissolved solids (ppm),
z = mean depth (m).

If the scaling of harvest on total dissolved solids resembles the scaling of harvest on depth then $|j| = |k|$. Further separation of variables is possible because mean depth z is itself the ratio of two variables; volume and surface area. As a consequence the partial regression of total yield Q on mean depth z is exactly equivalent to the partial regression of Q on volume, controlled for area. To clarify the relation of total yield, lake morphometry, and dissolved solids we separated variables to obtain an equation constructed entirely of measurement variables:

(5) $Q = e^g A^q V^v T^j$

Some increase in explained variance can be expected relative to equation 3 if the exponents in equation 5 are heterogenous.

The second approach to reducing prediction limits was to increase the generality of the predictive equation by replacing an empirical parameter with a theoretical parameter, as recommended by Platt et al. (1981). To do this we focused on derivation of a theoretical exponent for the variable, lake volume, that explained the largest proportion of variation in equation 5. Total harvest Q was hypothesized to be proportional to the flux F of limiting material through volume V of a lake:

(6) $Q\alpha = C^\beta F^\gamma V^\delta$

C is conversion efficiency (kg harvested·kg^{-1} limiting material),
F is flux of limiting material (kg·km^{-2}·yr^{-1}),
V is lake volume (km^3).

The exponent of the dependent variable Q is set equal to unity for convenience. Assuming geometrical similarity of lakes within a geological formation (Wetzel 1975), exponents for the other variables follow by dimensional analysis (Bridgman 1922):

(7) $Q = C\,F\,V^{2/3}$

This model was developed from exploratory analysis of Ryder's (1965) data set, so a second data set (Jones and Hoyer 1982) was used to test model predictions.

TABLE 1. Regression of area–specific fish harvest (H = kg·ha^{-1}·yr^{-1}) against the morphoedaphic index (MEI = ppm·m^{-1}) of Ryder (1965). Confidence limits computed for lake with largest area (Superior), smallest area (Heming), and median area (La Ronge). Geometric mean MEI is 5.99 ppm·m^{-1}.

Analysis of variance

Source	*df*	Sum of squares	Mean square	*F*-ratio	*p*
Model	1	7.2240	7.2240	57.402	0.0001
Residual	21	2.6428	0.1258		
Total	22	9.8668			

Parameter estimates ($H = e^c \mathrm{MEI}^b$)

Parameter	*df*	Estimate	SE
c	1	0.32386	0.12870
b	1	0.4458	0.05884

95% Prediction limits around predicted harvest (H)

Lake	Lower limit	Predicted harvest	Upper limit	Upper/ lower
Superior	0.40	0.91	2.08	5.2
La Ronge	1.94	4.13	8.81	4.6
Heming	2.49	5.38	11.61	4.7

Methods

Data reported by Ryder (1965) were converted to metric units for the analysis. Equation (1) was converted to linear form:

(8) $\log_e H = \log_e b + c \log_e \mathrm{MEI}$.

Regression estimates were calculated using the SAS package of routines. Regression estimates of parameters in equations 3, 4, and 5 were also obtained by linearizing the equation, then using least squares regression to estimate parameters. Prediction limits were calculated from equation 5.22 in Seber (1977), using the SAS package of routines. Prediction limits were calculated for the lake with the largest area (Superior), smallest area (Heming), and median area (La Ronge) in Ryder's data set.

Results

After conversion of data in Ryder (1965) to metric units, the regression estimates of the morphoedaphic equation are:

(9) $H = 1.3825\ \mathrm{MEI}^{0.4458}$

H = kg·ha^{-1}·yr^{-1},
MEI = ppm·m^{-1}.

This equation differs from the equation in metric units reported by Ryder et al. (1974). Equation 9 can be checked for consistency with equation 2 by algebraic conversion. The exponent in equation 2 is dimensionless, and so remains unchanged by change in units. The dimensions of the transfer coefficient depend on the exponent, which is used to convert the transfer coefficient to metric units as follows:

$$c = 2.094 \cdot (0.4536\ \mathrm{kg \cdot lb^{-1}}) \cdot (2.47\ \mathrm{acre \cdot ha^{-1}}) \cdot (0.3048\ \mathrm{m \cdot ft^{-1}})^{0.4461}$$

$$c = 1.3815\ \mathrm{kg \cdot ha^{-1} \cdot yr^{-1} \cdot ppm^{-0.4461} \cdot m^{0.4461}}.$$

The use of 2.47 acre·ha^{-1} can seem counter-intuitive, but it must be remembered that the goal is to convert acre^{-1} to hectare^{-1}. After algebraic conversion, equation 2 becomes:

TABLE 2. Regression of annual fish harvest (Q = kg·yr^{-1}) against area (A = ha) and the morphoedaphic index (MEI = ppm·m^{-1}) of Ryder (1965).

Analysis of variance

Source	*df*	Sum of squares	Mean square	*F*-ratio	*p*
Model	2	111.557	55.779	423.4	0.0001
Residual	20	2.6345	0.1317		
Total	22	114.192			

Parameter estimates ($Q = e^g A^h \text{MEI}^i$)

Parameter	*df*	Estimate	SE	Sum of squares
g	1	0.4772	0.6254	3966.258
h	1	0.9895	0.04178	108.244
i	1	0.4305	0.08583	3.3131

95% Prediction limits around predicted harvest Q' (Mg·yr^{-1})

MEI	Lower limit	Predicted harvest	Upper limit	Upper/ lower
Superior	3231	7525	17528	5.4
La Ronge	222	483	1055	4.8
Heming	0.60	1.46	3.55	5.9

TABLE 3. Regression of annual fish harvest (Q = kg·yr^{-1}) against area (A = ha), mean depth (z = m), and total dissolved solids (T = ppm).

Analysis of variance

Source	*df*	Sum of squares	Mean square	*F*-ratio	*p*
Model	3	111.8657	37.2886	304.61	<0.0001
Residual	19	2.3259	0.1224		
Total	22	114.1916			

Parameter estimates ($Q = e^g A^m V^n T^j$)

Parameter	*df*	Estimate	SE	Sum of squares
g	1	−1.535	0.9777	3966.258
q	1	1.588	0.1561	108.244
s	1	−0.5606	0.1162	2.8883
j	1	0.2940	0.1201	0.73344

95% Prediction limits around predicted harvest Q' (Mg·yr^{-1})

Lake	Lower limit	Predicted harvest	Upper limit	Upper/ lower
Superior	3158	7173	16289	5.2
La Ronge	221	471	1002	4.5
Heming	0.65	1.55	3.66	5.6

(10) $H = 1.3815\ \text{MEI}^{0.4461}$,

where H now has units kg·ha^{-1}·yr^{-1} and MEI now has units of ppm·m^{-1}. The parameters obtained by algebraic conversion (equation 10) are the same as the parameters obtained by regression (equation 9), to within three significant figures. The correct transfer coefficient in metric units (1.38) is 43% greater than the metric coefficient (0.966) reported by Ryder et al. (1974).

Table 1 shows the prediction limits around equation 9. We recommend caution in using these statistics because equation 1 results in an artificial correlation that inflates the explained variance. As a result, the prediction limits in Table 1 are underestimates of the true limits.

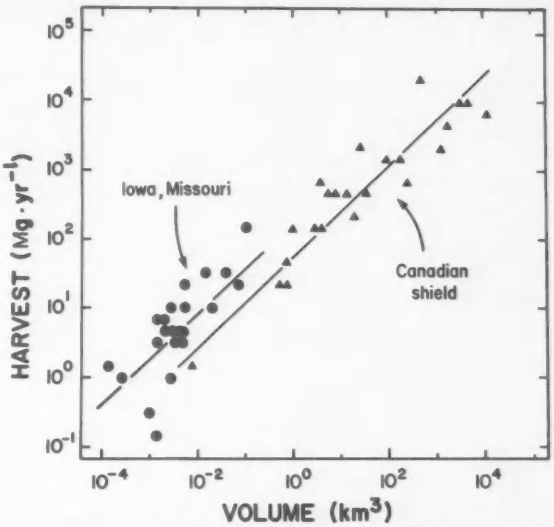

FIG. 1. Fish harvest in relation to lake volume. Triangles denote data from Ryder (1965). Circles denote data from Jones and Hoyer (1982). Lines show theoretical relation of harvest to lake volume within each set of data.

Underestimation can be remedied by obtaining least squares estimates of the parameters in equation 3. With parameters estimated from Ryder's (1964) data, equation 3 becomes:

(11) $Q = 2.26\, A^{0.99}\, \text{MEI}^{0.43}$.

Table 2 shows prediction limits for equation 11. These limits have expanded relative to limits based on equation 9 because apparent explained variance due to artificial correlation has been removed. The morphoedaphic index explains 55.7% (3.3131/(114.192 − 108.244)) of the residual variation in harvest after the effects of area are considered (Table 2) compared with the apparent explained variance of 73% (7.224/9.8668 Table 1) using equation 9. Similar results were obtained by Youngs and Heimbuch (1982) using a different set of lakes, including some used by Ryder (1965).

Ryder's morphoedaphic equation assumes that harvest scales with mean depth in the same way that it scales with dissolved solids. This assumption can be tested by separating variables, as in equation 4. The regression estimates for this equation are:

(12) $Q = 2.77\, A^{1.03}\, T^{0.29}\, z^{-0.56}$.

The absolute value of j did not differ significantly from the absolute value of k. The t-ratio is

$$(0.5606 - 0.2940)(0.1201^2 + 0.1162^2)^{-1/2} = 1.60,$$

which has an associated probability of 0.13 and 19 degrees of freedom. The two exponents, while not significantly different in this test, might differ significantly in a more sensitive test based on a larger number of cases.

This result suggested that further separation of variables, as in equation 5, could decrease the unexplained variance and thus narrow the confidence limits around predicted values. Table 3 shows the mean square error of this regression, along with the standard errors of the estimates of all four parameters in equa-

tion 5. Separation of variables increased the residual explained variance from 55.7% (SS = 3.3131 Table 2) to 60.9% (SS = 2.888 + 0.7334 = 3.62 Table 3). The decrease in mean squared error narrowed the spread between the upper and lower prediction limits (compare Table 2 with Table 3). The reduction in the mean squared error and in the prediction limits was, however, small. Separation of variables did little to improve the empirical estimate of fish harvest.

To test the theoretical model (equation 7) we compared the observed with the theoretical exponent relating fish harvest to lake volume. This test required backcalculation of lake volume from values of surface area and mean depth reported by Ryder (1965). The regression estimates were:

$$(13)\quad Q = 6.9 \times 10^4\, V^{0.60}$$

$$r^2 = 0.86,\ n = 23,\ p < 0.0001,$$
error mean square (EMS) = 15.697.

The observed (0.60) and expected (0.67) exponents did not differ significantly ($t = 1.35$, $df = 21$, $p = 0.10$).

The theoretical model can be further tested by comparing predicted and observed exponents relating fish yield to lake area. The relation between area A_o (ha) and volume V (km^3) in Ryder's (1965) data set is:

$$(14)\quad V = 3.6 \times 10^{-6}\, A_o^{1.32}.$$

The two parameters in this equation were estimated by least-squares regression. The variance explained by the regression was 97%. Substituting into equation (13):

$$(15)\quad Q = 6.9 \times 10^4\, (3.6 \times 10^{-6})^{0.60} A_o^{(1.32)(0.60)} \text{ or}$$

$$(16)\quad Q = 37.5\, A_o^{0.79}.$$

The observed relation between fish yield and surface area, as estimated by least squares regression, is:

$$(17)\quad Q = 20.9\, A_o^{0.84}$$

The variance explained by regression was 95%. The observed (0.84) and predicted (0.79) exponents did not differ significantly ($t = 1.21$, $df = 22$, $p = 0.12$).

The observed exponent differs significantly from unity ($t = 3.72$, $df = 22$, $p = 0.0006$). The theoretical scaling of fish harvest with lake volume correctly predicted that the exponent relating surface area to harvest will be less than unity, and hence that harvest per unit area will be less in large lakes than in small lakes.

The theoretical scaling of fish harvest against lake volume was then tested with a second data set, annual fish harvests from lakes and reservoirs in Missouri and Iowa (Jones and Hoyer 1982). If the theoretical scaling is correct, then harvests in this set of data should also be proportional to volume to the 2/3 power. Because the growing season is longer in the more southerly lakes, the transfer coefficient (the product of the flux coefficient F and the transfer efficiency C) should be greater in the southerly lakes than in the northerly lakes (Schlesinger and Regier 1982). The observed scaling of fish harvest against lake volume in the southerly lakes was:

$$(18)\quad Q = 4.0 \times 10^5\, V^{0.77}$$

$$r^2 = 0.61,\ n = 22,\ p < 0.0001,\ \text{EMS} = 21.474.$$

The observed (0.77) and theoretical (0.67) exponents did not differ significantly ($t = 0.77$, $df = 21$, $p = 0.22$). Analysis of covariance showed that the slopes are homogeneous ($F = 1.61$, $df = 1,41$, and $p = 0.21$). The transfer coefficient ($4.0 \times 10^5\ kg \cdot km^{-0.77} \cdot yr^{-1}$) was significantly greater than that of the more northerly lakes ($F = 3.64$, $df = 1,42$, and $p = 0.032$ in a one-tailed test). Figure 1 shows theoretical lines with a slope of two-thirds fit through both sets of data. Figure 1 does not show regression lines.

The flux model (equation 7) can be used to reduce prediction limits around estimates of potential harvest of fish. If the model is correct then:

$$(19)\quad Q = k\, V^{2/3},$$

which has only one empirical coefficient, k. This transfer coefficient can be estimated as the average value of $Q\, V^{-2/3}$ in a set of geometrically similar lakes. The average value of k for the set of lakes of Ryder (1965) is 8.3×10^4 and hence:

$$(20)\quad Q = 8.3 \times 10^4\, V^{2/3}.$$

The standard error around this estimate of k is 1.9×10^4, resulting in an upper confidence limit ($1.2 \times 10^5\, V^{2/3}$) that is 2.8 times the lower limit ($4.4 \times 10^4\, V^{2/3}$). This range is substantially less than the range around empirical estimates (Tables 1, 2, and 3) and assumes that the theoretical exponent of two-thirds is correct. The prediction limits around the theoretical line do not expand with increasing distance from the mean of the dependent variable, lake volume. This stands in contrast to the prediction limits around a regression line fit through the mean value of the dependent variable.

Discussion

Empirical equations for predicting fish stock or potential harvest from lakes are typically compared on the basis of explained variances (Ryder 1965; Hanson and Leggett 1982). These comparisons can be misleading when explained variances are inflated by artificial correlation, a relatively minor problem in the case of Ryder's morphoedaphic index. In other cases, however, artificial correlation of dependent and independent variables could be a serious problem.

Prediction limits have not been reported for morphoedaphic estimates of potential fish harvest. Analysis of Ryder's (1965) lake data showed that prediction limits were underestimated because of artificial correlation. After removal of artificial correlation, the upper 95% prediction limits of morphoedaphic estimates were 4.8–5.9 times the lower 95% prediction limit. The prediction limits of fish harvests were much wider than one might expect from examining the explained variances, which were well over 95% for the multiple regressions (Table 2, 3). Wide prediction intervals reflect the fact that errors around exponential estimates are multiplicative rather than additive. As a result, little reduction in the width of prediction intervals can be expected unless the residual mean error can be reduced substantially.

In two sets of data the exponent relating fish harvest to lake volume was predicted by assuming that fish harvest depends on the flux of a limiting material through a lake. The flux model predicted that the harvest of fish from large lakes will exceed the harvest of fish from small lakes, but that small lakes will yield more fish per unit of surface area. The prediction is consistent with the conventional wisdom that harvest per unit area of small lakes cannot be used to predict harvest per unit area of larger lakes (Rounsefell 1946; Carlander 1955). The predicted scaling ($V^{2/3}$) was consistent with the observed allometry of harvest from lakes at two different latitudes in North America.

Equation 20 provides an alternative model to the morphoedaphic equation for predicting fish yield from lakes or reservoirs of known size. In the interest of consistency with previous work we recommend that the morphoedaphic estimate continue to be calculated, using either Ryder's (1965) equation or our equation 9. If Ryder's 1965 equation is used then conversion to metric units is made after computing yield in pounds per acre-year. We also recommend that prediction limits be reported. For this purpose equation 11 should be used if feasible, because it is free of artificial correlation. Unfortunately, the calculations are cumbersome. One solution is to enter Ryder's data into a computer and then allow a packaged routine to generate confidence limits for a lake or reservoir of known volume and total dissolved solids. A simpler but less desireable alternative is to use the residual mean square and the mean MEI in Table 1 to compute prediction limits based on equation 9. The resulting limits will be narrower than limits calculated from equation 11, which is free of artificial correlation. We do not go so far as to recommend complete replacement of empirical models with our theoretical model (equation 20). We do note that predicted yields with confidence limits are easily calculated from equation 20. The only variable needed is volume, a known variable or one which is always projected for new reservoirs established as hydroelectric projects. We recommend that predictions derived from equation 20 be labelled as theoretical, and considered supplementary to empirical predictions until the theoretical model is corroborated in several new reservoirs.

A reviewer of an earlier version of this paper pointed out that the method we used to eliminate artificial correlation could, in other circumstances, generate artificial correlation. In the analysis of fish yields we recovered a measurement variable (Q = annual fish yield of a lake) from a derived variable (H = annual fish yield per unit area of a lake). This procedure can easily generate a derived variable from a measurement variable. For example, multiplying benthic biomass per unit sampling area (the ratio of two measurement variables) by the number of such sampling areas per lake to obtain total benthic biomass per lake generates a derived variable having an artificial correlation with lake area. We therefore offer a simple rule for avoiding artificial correlation by determining whether or not the dependent variable in a regression has been derived from other variables. If any of the "parent" variables appear either directly or in derived form as independent variables, then the variation explained by regression will be inflated by artificial correlation.

We were also challenged to comment on empirical and theoretical approaches to ecological problems (Peters 1986; Lehman 1986). The first part of our analysis (through equation 5) is clearly in the "empirical" tradition that seeks improved equations to predict highly aggregated variables such as fish yield. What we found was that little further improvement was possible with the data available, if improvement was defined as an appreciable narrowing of prediction limits. We then adopted a more theoretical approach aimed toward an understanding of why fish yield should be an allometric function of lake size. The theoretical analysis of fish yield (equation 6 and 7) is in the spirit of the tradition that seeks improved understanding based on process and mechanism. Our concept at its most parsimonious suggests that biological activity in a volume of water is limited by the flux of material through some critical surface. The concept can be traced to the work of Grimard and Jones (1982); Ostrofsky (1978); and Vollenweider (1968) on trophic upsurge in new reservoirs. We have shown that the concept produces predictions corroborated by additional data that substantially narrow prediction limits around an otherwise empirical equation.

Conclusions

(1) The morphoedaphic equation in metric units (Ryder et al. 1974) is inconsistent with the original equation proposed by Ryder (1965). The correct transfer coefficient (1.38) should be used to calculate morphoedaphic estimates of fish yield in the metric system.

(2) Artificial correlation related to the contribution of area to both the dependent and independent variable in the original formulation of the MEI model inflates the explained variance in the regression of fish yield on the morphoedaphic index.

(3) After removal of artificial correlation, the upper 95% prediction limits for the morphoedaphic equation are 4.8–5.9 times the lower prediction limit. The width of the intervals reflects the fact that errors around the estimate of an exponent have a multiplicative effect on predictions.

(4) Error mean square and prediction limits can be reduced by separating variables in equations based on composite indices. In the case of the morphoedaphic index, separation of variables reduced EMS and prediction limits by relatively small amounts.

(5) Theoretical scaling of fish harvest on lake volume reduced the upper 95% prediction limit to 2.8 times the lower limit. The theoretical scaling was consistent with the known tendency of small lakes to yield more fish per unit area than large lakes. The theoretical scaling correctly predicted the exponent relating fish harvest to lake area. The theoretical scaling correctly predicted the exponent relating fish harvest to lake volume in a set of data that was not used to develop the theoretical scaling.

(6) The theoretical scaling needs further testing against independent data. Further reduction in prediction limits may be possible by developing a theoretical scaling of fish harvest on total dissolved solids.

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Terrestrial and In-Lake Contributions to Alkalinity Budgets of Drainage Lakes: An Assessment of Regional Differences

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Using two independent procedures, we estimate relative contributions of in-lake alkalinity generation to total basin alkalinity budgets for drainage lakes in selected regions of the Eastern United States. The first method is based on the assumption of approximately equal areal rates of alkalinity production by watersheds and lakes, and uses watershed area to lake area ratio as a direct estimator of relative akalinity contributions. The second approach is based on existing models for in-lake alkalinity generation estimated from sulfate retention. Projections from the two methods are comparable, and show that for most drainage lakes in Northeastern United States, Southern Blue Ridge Province, and Upper Midwest, in-lake alkalinity generation is a minor contributor to net basin alkalinity production, contributing ≤7% of total net basin alkalinity production in typical watersheds in any of the regions. The close agreement between the two procedures lends support to the use of watershed area to lake area ratio as a convenient estimator of relative alkalinity sources requiring only map-derived parameters. Regional assessment of current or future effects of acidic deposition on surface water chemistry in the Northeastern United States, the Southern Blue Ridge Province or other areas dominated by drainage lakes with high watershed to lake area ratio would not appear to be seriously comprised by use of models limited to consideration of terrestrial processes. In areas such as the Upper Midwest which are characterized by high proportions of seepage lakes or for lakes with long hydrologic residence times, in-lake processes and hydrologic influences must be considered.

En employant deux procédures indépendantes, nous estimons les apports relatifs de substances alcalines produites dans les lacs aux bilans de l'alcalinité totale des bassins pour ce qui est des lacs de drainage dans certaines régions de l'est des États-Unis. La première méthode est basée sur l'hypothèse de taux surfaciques de production de substances alcalines à peu près égaux par les bassins versants et les lacs, et fait appel au rapport entre la superficie du bassin versant et celle des lacs comme estimateur direct des apports relatifs de substances alcalines. La deuxième approche est basée sur des modèles existants de production de substances alcalines dans les lacs estimée d'après la rétention de sulfates. Les prévisions faites à partir des deux méthodes sont comparables et montrent que, pour la plupart des lacs de drainage situés au nord-est des États-Unis, dans la partie sud de la chaîne Blue Ridge et dans le midwest supérieur, la production de substances alcalines dans les lacs contribue de façon mineure à la production nette de substances alcalines dans les bassins, soit pour 7 % de la production totale nette de substances alcalines des bassins dans les bassins versants caractéristiques de l'une ou l'autre de ces régions. L'étroite concordance entre les deux procédures vient corroborer l'utilisation du rapport entre la superficie du bassin versant et celle des lacs comme estimateur approprié des sources relatives de l'alcalinité qui n'exige que des paramètres tirés des cartes. L'évaluation à l'échelle régionale des effets actuels et futurs du dépôt d'acides sur la chimie des eaux de surface dans le nord-est des E.-U., dans la zone sud de la chaîne Blue Ridge ou dans d'autres secteurs dominés par des lacs de drainage, où le rapport entre la superficie du bassin versant et celle des lacs est élevé, ne semblerait pas gravement compromise par l'utilisation de modèles qui se limitent à l'examen de processus terrestres. Dans des secteurs comme le midwest supérieur qui sont caractérisés par des proportions élevées de lacs d'infiltration ou, dans le cas de lacs où le temps de séjour de l'eau est long, il faut tenir compte des processus qui se déroulent dans les lacs et des influences hydrologiques.

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During the past decade, a number of investigators have shown that in-lake processes contribute significantly to lake alkalinity budgets in diverse geographic regions (e.g. Cook and Schindler 1983; Schindler et al. 1986; Baker et al. 1985, 1986a; Lin and Schnoor 1986). Work to date has described widespread occurrence of in-lake alkalinity generation (IAG) and has defined the importance of IAG to alkalinity budgets in precipitation-dominated seepage lakes and in lakes with long hydrologic residence times. Most conceptual and mathematical models used to forecast future effects of acidic precipitation on surface water chemistry, however, are limited to consideration of terrestrial processes (e.g. Galloway et al. 1983, NRC 1984; Goldstein and Gherini 1984; Cosby et al. 1985; Rustad et al. 1986). The importance of IAG to basin alkalinity budgets in lakes with short residence times has not been evaluated, and remains controversial (Schafran and Driscoll 1987; Shaffer et al. 1988, (cf. Schindler 1986).

Shaffer et al. (1988) compiled data on terrestrial and in-lake alkalinity production and determined that for the low-alkalinity systems examined, areal lake and terrestrial alkalinity produc-

FIG. 1. Location map of study regions in the Eastern United States.

TABLE 1. Comparison of terrestrial and in-lake alkalinity generation rates in softwater (pH ≤7.0, alk ≤200 $\mu eq \cdot L^{-1}$) forested systems in North America and Europe. Data summarized from Shaffer et al. (1988).

Location	No. of sites	Areal net alkalinity generation ($meq \cdot m^{-2} \cdot yr^{-1}$) Median	Mean	Range
In-lake production	11	88	99	22 – 240
Watershed production				
wet/bulk deposition only	20	69	89	20 – 235
dry deposition increment	10	34	35	13 – 48

tion rates were roughly equal. If equal areal rates of alkalinity production are assumed, watershed to lake area ratio can be used as a convenient estimator of IAG contributions to total basin alkalinity production. This approach is not as rigorous as the development of input–output budgets (e.g. Wright 1983; Schindler et al. 1986) or the retention models of Baker et al. (1986b, 1988) and Kelly et al. (1987). The watershed to lake area approach, however, can be applied using only map-derived watershed and lake areas, rather than the site–specific long-term hydrochemical monitoring or hydrologic and morphometric data required by the other methods, and is thus potentially applicable to all drainage watersheds in a region.

In this manuscript, we estimate the relative importance of in-lake processes to total basin alkalinity budgets at regional scales for the Eastern United States, using the watershed area to lake area (WA:LA) approach with data from the U.S. Environmental Protection Agency's Eastern Lake Survey (ELS) (Linthurst et al. 1986; Kanciruk et al. 1986). We also use the IAG models of Baker et al. (1986b, 1988) and Kelly et al. (1987) to compute sulfate retention for lakes in the same regions. We complete our analysis by comparing projections from the IAG models with those of the WA:LA approach.

Study Area and Data Sources

The study regions used here represent regions or subregions of the ELS (Linthurst et al. 1986; Kanciruk et al. 1986). The three regions considered are the Northeastern United States (NE), Upper Midwest (UMW), and Southern Blue Ridge Province (SBRP) (Fig. 1). In addition, the subregion of the NE with the lowest average surface water alkalinity, roughly coincident with the Adirondack Mountains of New York, is included with the NE regional analyses and is assessed separately. ELS data are extrapolated to regional population distributions from data for 1452 lakes sampled in the three regions under consideration (n = 763 in NE, 587 in UMW, and 102 in the SBRP) and selected as a stratified sample of all lakes delineated on U.S. Geological Survey 1:250 000 topographic maps (nominal area ≥4 ha) in the survey regions (Linthurst et al. 1986). Lakes were characterized by ELS as drainage (having a well-defined surface outlet), impoundment (man-made), seepage (no inlet or outlet), or closed (inlet, no outlet) based on inspection of 7.5- or 15-min topographic maps and onsite inspection during lake sampling (Linthurst et al. 1986). Impoundments are included with drainage lakes in all calculations and discussions. Mapped topographic divides were used to define watershed areas. We assumed that differences between topographic and hydrologic boundaries are minor for most drainage systems. Such differences, however, might be problematic in areas with little relief, and are often severe in seepage lake watersheds (e.g. Baker et al. 1986a; Lin and Schnoor 1986). Due to the uncertain hydrologic boundaries and resulting uncertainty in actual WA:LA ratios for seepage lakes, we estimated IAG contributions to alkalinity budgets only for drainage lakes.

Table 1 summarizes data compiled by Shaffer et al. (1988) and includes alkalinity generation rates for low alkalinity watersheds (20 sites) and lakes (11 sites) in North America and Europe. Data are based on results of whole lake or watershed studies. Areal IAG rates from laboratory or field micro/mesocosm studies are not included with these data, but are generally consistent with rates from whole system studies (Rudd et al. 1986; Kelly et al. 1987). For both watershed alkalinity generation (WAG) and IAG rates, data are used as presented by the original authors, and represent net alkalinity production. Watershed alkalinity generation rates are based on wet/bulk deposition data; terrestrial rates adjusted for dry deposition are also included, based on data from 10 sites reporting estimates of dry deposition.

The conclusion of Shaffer et al. (1988) of equal IAG and WAG rates represents a best estimate of relative rates based on available data. Existing data do not provide a statistically representative set of watersheds and lakes, and the number of coupled lake and watershed sites is too sparse to allow a rigorous analysis of relative IAG and WAG rates. Despite this uncertainty, it seems unlikely that the actual ratio of IAG:WAG deviates substantially from unity in most cases. Therefore, using a ratio of one does not seriously detract from our use of WA:LA ratios as a rough estimator of the relative importance of IAG. To the extent that actual IAG:WAG ratios deviate from unity, our calculations would underestimate IAG contributions to alkalinity budgets in cases where IAG:WAG is >1, and would overestimate the role of IAG if the ratio is less than unity.

Data Analysis

IAG Contributions Estimated from Watershed Area–Lake Area Data

If rates of alkalinity production are known for a lake and its surrounding watershed, the portion of alkalinity generated by in-lake processes can be computed as:

TABLE 2. Physical characteristics of lake watersheds in selected areas of the Eastern United States and projected contributions of IAG to total basin alkalinity. Descriptive physical data for lakes from Linthurst et al. (1986).

Region[a]	Lake type[b]	Regional lake population No.	Regional lake population %	Median WA:LA ratio[c]	% of lakes with WA:LA ≤4.0	Median % of alkalinity production by IAG[d]	% of lakes with ≥20 total alkalinity from IAG[e]
Northeast	Drainage	6288	89	13.0	10.3	7.1	10
	Seepage	478	7	3.8			
	Closed	330	5	10.0			
Adirondacks	Drainage	1091	85	14.0	9.7	6.6	10
	Seepage	110	8	5.1			
	Closed	89	7	7.1			
Upper Midwest	Drainage	4404	52	14.0	16.0	6.6	16
	Seepage	3646	43	4.7			
	Closed	451	5	7.4			
Southern Blue Ridge Province	Drainage	250	97	41.0	<0.5	2.4	<0.5
	Seepage	0	0	—			
	Closed	7	3	ND			

[a]Regions are identified in Fig. 1.
[b]Lake types are as defined by Linthurst et al. (1986), and discussed in text. Impoundments are included with drainage lakes.
[c]WA:LA is watershed area to lake area; watershed refers only to the terrestrial portion of a lake/watershed system.
[d]Computed using equation (2).
[e]Equal to percent of lakes with WA:LA ratio ≤4.0, i.e. with lake covering at least 20% of total basin area.

$$(1)\quad \%ALK_{IAG} = \frac{\text{lake area} \times IAG \times 100}{(\text{lake area} \times IAG) + (\text{watershed area} \times WAG)}$$

Assuming WAG = IAG on an areal basis reduces equation (1) to:

$$(2)\quad \%ALK_{IAG} = \frac{\text{lake area} \times 100}{\text{lake area} + \text{watershed area}} = \frac{100}{1 + WA:LA}$$

(Watershed area as used here refers only to the terrestrial portion of a lake/watershed system.) Using WA:LA data for individual lakes sampled in the ELS (Linthurst et al. 1986; Kanciruk et al. 1986), we estimated the fraction of total basin alkalinity production by IAG using equation (2). In addition, using equation (2) with ELS distributions of WA:LA for the drainage lake population in each region, we estimated IAG contributions to total alkalinity for lakes with median WA:LA in each region, along with the fraction of lakes in each region where $\%ALK_{IAG}$ is ≥20% of total alkalinity (i.e. the fraction of lakes with WA:LA ≤4.0). We also used the ELS data set to identify areas with large populations of seepage/closed lakes, in which estimates of IAG based on drainage lake WA:LA ratios might seriously misrepresent the overall regional importance of IAG.

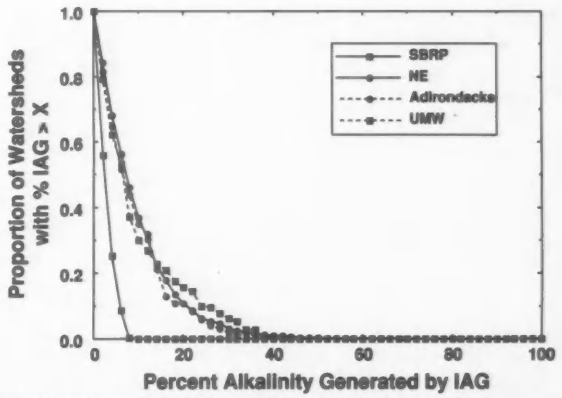

FIG. 2. Estimated cumulative distribution of in-lake alkalinity generation, as a percentage of total basin alkalinity production, for drainage lake populations in ELS regions. Estimates of IAG contributions are based on the WA:LA approach of Shaffer et al. (1988).

Estimates of Sulfate Retention

Using models for sulfate retention developed by Baker et al. (1986b, 1988) and by Kelly et al. (1987), we estimated sulfate retention within lakes to be:

$$(3)\quad \text{Percent sulfate retention} = \frac{100 \times k_{SO_4}}{(Z/t_w) + k_{SO_4}}$$

where K_{SO_4} = sulfate mass transfer coefficient (metres per year), Z = mean lake depth (metres), and t_w = water residence time (years). Calculations of sulfate retention were made for individual lakes in each region, and also using median and 80th percentile values of Z and t_w for lake populations in individual regions of the Eastern United States, taken from ELS data (Linthurst et al. 1986, Kanciruk et al. 1986). Median data provide a measure of sulfate retention in "typical" lakes of each region, whereas the 80th percentile data were included to assess the degree of sulfate retention in those systems where high retention is expected due to long hydrologic residence times. Based on data summarized by Baker et al. (1986b) and Kelly et al. (1987), 0.5 $m \cdot yr^{-1}$ was used as the mass transfer coefficient for the sulfate ion.

As a final step in data analysis, the projected percent sulfate retention was used with lake sulfate concentration to estimate the effect of in-lake sulfate retention on lake sulfate concentrations:

$$(4)\quad \Delta SO_4 = SO_{4_{lake}} \times \left(\frac{100}{100 - \%SO_4\, rtn} - 1 \right),$$

where ΔSO_4 is the projected decrease in lake SO_4 (micro equivalents per litre) and corresponding increase in alkalinity that has resulted from in-lake sulfate reduction; $SO_{4_{lake}}$ is measured

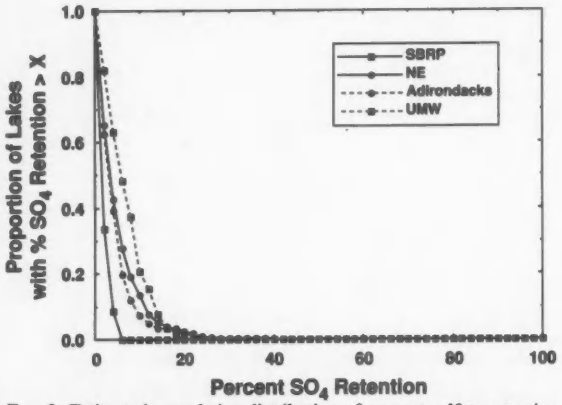

FIG. 3. Estimated cumulative distribution of percent sulfate retention within lakes for drainage lake populations in ELS regions. Estimates of sulfate retention are based on the IAG models of Baker et al. (1986b, 1988) and Kelly et al. (1987).

lake sulfate concentration or the median or 80th percentile sulfate concentration for a region; and $\%SO_4$ retention data are taken from equation (3). Results using this calculation, which is based on lake sulfate concentration, are equivalent to changes in alkalinity computed using the neutralization equation (equation 6) of Kelly et al. (1987), which is based on deposition loading and lake outflow.

Results and Discussion

IAG Estimates Based on WA:LA

Table 2 describes regional differences in hydrologic lake type and WA:LA ratio for lakes in the Eastern United States, and summarizes the estimated relative regional importance of IAG, based on WA:LA data and expressed as an estimated percent of total net alkalinity production from IAG. The data identify major regional differences in abundance of different lake types, along with substantial differences in WA:LA. The NE is dominated by drainage lakes and impoundments (89% of all lakes) with high WA:LA ratios (median = 13:1). Assuming equal rates of WAG and IAG, in-lake sources are estimated to contribute only 7% of total basin alkalinity to typical systems in the region, and to provide ≥20% of the total alkalinity in only 10% of NE lakes. Seepage and closed basin lakes account for only 11% of lakes in the region, so the drainage lake summary data describes most NE standing surface waters. For the Adirondack Mountains, lake type distributions, WA:LA ratios, and projected IAG contributions to lake/watershed alkalinity budgets are comparable with those of the region as a whole.

Watershed-to-lake area distributions for drainage lakes in the UMW are not substantially different from those of lakes in the NE. The median WA:LA ratio is again high, although WA:LA is ≤4:1 for a higher fraction of lakes in the UMW than in the NE. The estimated median in-lake contribution to net basin alkalinity budgets is 7%, with IAG contributing more than one-fifth of total alkalinity in an estimated 16% of drainage lakes. The principal difference between lakes in the two regions lies in the relative abundance of drainage and seepage/closed lakes. Only 52% of lakes in the UMW are drainage systems, most of the balance are seepage lakes. Based on the importance of precipitation-dominated hydrology and chemistry in seepage lakes in the UMW (Eilers et al. 1983; Lin and Schnoor 1986), IAG is probably a major contributor to alkalinity budgets for a sizeable fraction of lakes in the region. Groundwater contributions are also likely to be a significant factor controlling surface water chemistry in the region (Eilers et al. 1983; Rapp et al. 1985). For the region as a whole, there is an apparent dichotomy in alkalinity sources to lakes. Roughly half the lakes are drainage systems projected to be dominated by terrestrial alkalinity sources, whereas over 40% of the lake population is seepage lakes, in which in-lake processes typically contribute a significant fraction or a majority of alkalinity production.

Drainage lakes and reservoirs dominate surface waters in the SBRP, comprising 97% of water bodies in the region (90% reservoirs). WA:LA ratios in the region are very high, with a median ratio of 41:1 and a maximum greater than 5000:1. Accordingly, IAG is of only very minor importance in alkalinity budgets of surface waters in the region. The estimated median contribution of IAG is only 2% of total alkalinity, and it probably accounts for more than 20% of total alkalinity in fewer than 0.5% of lakes in the SBRP.

Cumulative frequency distributions of estimated IAG contributions to alkalinity budgets (Fig. 2) emphasize the low IAG contributions to most lake systems in these regions. In about two-thirds of the watersheds in the NE, the Adirondacks, and UMW, IAG is estimated to supply <10% of total alkalinity. It is projected to supply more than 30% of alkalinity in only 2–3% of watersheds in the NE and Adirondacks and 6% in the UMW, and more than 40% in less than one-half percent of lakes >4 ha. In the SBRP, the dominance of terrestrial alkalinity sources is extreme; IAG is projected to supply <4% of alkalinity to about 75% of lake systems; the maximum projected

TABLE 3. Estimated sulfate retention in drainage lakes in the Eastern United States.

Region	Population statistic	Depth (m)*	Retention time (yr)*	% SO_4 retention	Median lake SO_4 ($\mu eq \cdot L^{-1}$)*	ΔSO_4 ($\mu eq \cdot L^{-1}$)
Northeast	Median	4.2	0.2	2.3**	115	2.7
	80%	8.3	0.7	4.1		4.9
Adirondacks	Median	5.6	0.2	2.0	119	2.4
	80%	10.1	0.6	2.8		3.4
Upper Midwest	Median	5.6	0.5	4.1	57	2.4
	80%	10.9	1.6	7.0		4.3
Southern Blue Ridge Province	Median	4.8	0.1	1.0	32	0.3
	80%	12.5	0.3	1.3		0.4

*From Linthurst et al. (1986).
**Computed using equation (3).

TABLE 4. Estimated per cent of basin alkalinity derived from IAG in drainage lakes of the Eastern United States, based on sulfate retention data (Table 3).

Region	Population statistic	ΔSO_4 by IAG ($\mu eq \cdot L^{-1}$)	Estimated alkalinity from IAG ($\mu eq \cdot L^{-1}$)	Median alkalinity ($\mu eq \cdot L^{-1}$)	% of median alkalinity
Northeast	Median	2.7[a]	2.7 – 8.1[b]	158[c]	1.7 – 5.1
	80%	4.9	4.9 – 14.8		3.1 – 9.3
Adirondacks	Median	2.4	2.4 – 7.3	112	2.2 – 6.5
	80%	3.4	3.4 – 10.3		3.1 – 9.2
Upper Midwest	Median	2.4	2.4 – 7.3	360	0.7 – 2.0
	80%	4.3	4.3 – 12.9		1.2 – 3.6
Southern Blue	Median	0.3	0.3 – 1.0	250	0.1 – 0.4
Ridge Province	80%	0.4	0.4 – 1.3		0.2 – 0.5

[a]From Table 3.
[b]Assuming SO_4 retention = 33–100% of total IAG, i.e. that total IAG is 100–300% of IAG from sulfate retention.
[c]From Linthurst et al. (1986).

TABLE 5. Summary comparison of estimated in-lake alkalinity generation in drainage lakes of the Eastern United States, as a percentage of net basin alkalinity.

	% Total alkalinity from IAG	
Region	WA:LA estimate	SO_4 retention estimate
Northeast	6.6	1.7 – 5.1
Adirondacks	6.2	2.2 – 6.5
Upper Midwest	6.2	0.7 – 2.0
Southern Blue Ridge Province	2.3	0.1 – 0.4

IAG contribution in the region is only 13% of total basin alkalinity.

Estimates of IAG from Sulfate Retention Data

Estimates of sulfate retention by drainage lakes in the same regions are presented in Fig. 3. Results are consistent for projections for IAG, showing low in-lake sulfate retention by the great majority of drainage systems in all regions considered. Median retention in NE and Adirondack lakes is about 4%; retention is <10% for 85–90% of lakes considered in these regions, and exceeds 20% for <2% of lakes. Median retention in lakes of the UMW is moderately higher than in the NE, but is not projected to exceed 20% in any lakes. As was the case for IAG projections, estimated sulfate retention in the SBRP is very low, with <2% retention projected for two-thirds of lakes of the region, and a maximum estimated retention of only 6%.

Summary data in Table 3, based on median and 80th percentile data of population distributions for individual physical/chemical variables, are consistent with the distributions in Fig. 3. In addition, for both median and 80th percentile lakes, sulfate retention was low in all regions considered, ranging from 1% in the SBRP (median data) to only 7% for lakes in the UMW using 80th percentile data. If sulfate retention is expressed in terms of its effect on dissolved sulfate concentration, the projected effect of in-lake sulfate retention is a decrease in SO_4 concentration of only 0.3 – 4.9 $\mu eq \cdot L^{-1}$. Viewed in the context of uncertainty in SO_4 measurements and input–output budgets, the regional effect of in-lake sulfate retention in drainage lakes of these areas is negligible. However, two caveats should be noted. First, we reiterate that the data reported here represent sulfate retention rates for drainage lakes only. Second, sulfate retention in some individual lakes might deviate substantially from our projections, inasmuch as our estimates are based on an average value of k_{SO_4} which is expected to vary among individual lakes.

Table 4 provides a rough estimate of total IAG extrapolated from sulfate retention data in Table 3 and from summary data of Brezonik and Baker (1987), showing that SO_4 retention accounts for 33 to ≥100% of total IAG (i.e. that total IAG is 100 to 300% of sulfate retention on an equivalent basis). Using the most conservative estimate of IAG (i.e. that sulfate retention equals total IAG), we project total IAG to provide no more than 5 $\mu eq \cdot L^{-1}$ of alkalinity in typical lakes of the Eastern United States. This is equivalent to no more than 3% of median regional alkalinity. Furthermore, of necessity, this comparison uses measured lake alkalinity (which reflects terrestrial alkalinity yield), so it overestimates the importance of IAG on net alkalinity production budgets. Even using the "worst case" estimate (i.e. that SO_4 retention accounts for only 33% of total IAG and using 80th percentile lake data) we project IAG to provide only 1 – 15 $\mu eq \cdot L^{-1}$ of alkalinity in typical regional lakes. The IAG contribution to total alkalinity in these "worst" cases is <1% of median alkalinity in the SBRP, and no more than 9% of median alkalinity in the NE. The implication of these results is that even using the "worst-case" factors, IAG is projected to be a relatively small contributor to total lake/watershed alkalinity budgets for typical drainage lakes (≥4 ha) in the regions considered here.

General Discussion

We have used two independent estimators to assess the contribution of in-lake alkalinity processes to total alkalinity budgets in drainage lakes in several geographic regions of the Eastern United States. Results from the two methods provide consistent projections indicating a small relative contribution by IAG in most drainage lake systems in the NE, UMW, and SBRP (Table 5). WA:LA data indicate that only 2 – 7% of net basin alkalinity is derived from IAG in typical systems in the three regions, and projections based on sulfate retention are comparable, ranging from ≤1 to 7% for the three regions. Given our objective of making a preliminary assessment of IAG contributions to total alkalinity budgets, it is relatively unimportant to determine which of the two approaches is more accurate. Rather, the critical result is that both approaches project low

relative IAG contributions to total alkalinity production in most drainage lakes in the three regions under consideration.

Estimates based on watershed area to lake area relationships consider total alkalinity production for the basin. Estimates of alkalinity using percent sulfate retention are expressed as a percentage of lake alkalinity, which reflects alkalinity production by the lake but only alkalinity yield from the terrestrial system. Systems receiving acidic deposition will have terrestrial alkalinity yield (output) that is lower than production (output minus input) due to neutralization of acidic inputs, with the end result that estimates of IAG based on sulfate retention will overestimate IAG contributions to total alkalinity budgets.

The estimates of low IAG developed here are consistent with two additional lines of evidence concerning the Eastern United States. Our estimates of low sulfate retention in NE lakes are consistent with sulfur input–output budgets developed for lakes in the region by Rochelle and Church (1987), which show most lake/watersheds to be at or very near steady state for sulfur. Secondly, the low projections of IAG are consistent with the combination of short hydrologic residence times noted in Table 2, and the comments by Baker et al. (1986b, 1988) that IAG has little effect on surface water alkalinity in systems with residence times shorter than 1 yr.

Comparison of projections from the WA:LA approach and from the IAG models of Baker et al. (1986b, 1988) and Kelly et al. (1987) allows us to assess the utility of WA:LA ratios as an estimator of IAG contributions to total alkalinity budgets. The two approaches provide comparable projections, suggesting that either can be used with equivalent confidence, although there are conditions favoring use of one approach over the other. The Baker and Kelly models have an advantage over the WA:LA approach in that they provide for quantitative treatment of rates of IAG, and we suggest their use where the necessary hydrologic data are available. At regional scales or at sites with sparse data, however, the Baker and Kelly models are at a significant disadvantage because they require estimates or on-site collection of hydrologic data. The use of WA:LA ratios to estimate the relative role of IAG is simple, and the only data required can be readily obtained from topographic maps. Data presented here support use of WA:LA ratios as a convenient, widely applicable method for estimating sources of alkalinity production in lake/watershed systems.

The relative importance of IAG in net basin alkalinity budgets in most systems is largely determined by two factors; (1) absolute rates of IAG and (2) lake hydrologic variables (or perhaps more explicitly, the volume of water into which alkalinity is released, or the annual discharge per unit lake area). Rates of in-lake alkalinity production (expressed as k_{SO_4}) apparently vary over a fairly narrow range among lakes studied (Rudd et al. 1986; Kelly et al. 1987, Brezonik and Baker 1987), and in typical drainage lakes of the Eastern United States are probably comparable with rates measured in systems where IAG is a major contributor to total alkalinity (Brezonik and Baker 1987; Kelly et al. 1987). Hydrologic residence times of lakes, however, can vary greatly among regions. For example, at the Experimental Lakes Area in Ontario (ELA) and in many seepage lakes (e.g. Schindler et al. 1986; Lin and Schnoor 1986; Baker et al. 1986a), residence times are long and IAG dominates alkalinity budgets. By contrast, residence times in most drainage lakes of the Eastern United States are <1 yr, averaging only 1–3 mo in most drainage lakes of the NE, UMW, and SBRP (Linthurst et al. 1986). The relatively minor role estimated for IAG in drainage lakes of the Eastern United States is thus viewed as a consequence of short hydrologic residence times, rather than of low inherent rates of IAG. Alkalinity produced in the lakes is diluted into large volumes of water and its relative role in minimized. The importance of residence time is implicit in WA:LA based estimates of IAG, and is explicit in the models of Baker et al. (1986b, 1988) and of Kelly et al. (1987). Those authors concluded, and our findings agree, that in lakes with short hydrologic residence times (1 yr or less), in-lake processes have little net effect on sulfate or total alkalinity budgets.

Conclusions

For three regions of the Eastern United States, using two independent estimation procedures, data indicate that IAG is a minor contributor to net alkalinity budgets in most drainage lake systems. For the NE, UMW, and SBRP, in-lake processes are estimated to contribute no more than 7% of alkalinity in typical watershed/lake systems. These estimates describe the great majority of systems in the NE and SBRP, which are dominated by drainage lakes and impoundments, but only about one-half the watersheds in the UMW, where 47% of lakes are seepage or closed lakes expected to have significant IAG and/or groundwater contributions to alkalinity budgets.

Based on the first conclusion, it appears that regional assessments of current or predicted future effects of acidic deposition on surface water chemistry, at least in the NE and SBRP, are not seriously compromised by use of models limited to consideration of terrestrial processes. In areas with a high proportion of seepage lakes or of lakes with long hydrologic residence times, or for individual systems with long residence times in any geographic area, in-lake processes and hydrologic influences must be considered in detail.

The two approaches used here, the IAG models of Baker et al. (1986b, 1988) and Kelly et al. (1987) and the watershed area to lake area approach of Shaffer et al. (1988), provide very comparable estimates of IAG contributions to alkalinity budgets, with slightly higher estimates of IAG from the WA:LA model. The consistency between the two approaches, along with the consistency between them and other lines of evidence regarding the role of IAG in the regions under consideration, suggests that either can be used with confidence as a means of estimating the importance of IAG. The selection of either approach should be based on availability of hydrologic data for the system or region under consideration. We suggest use of the Baker or Kelly models if hydrologic data can be obtained. The WA:LA approach provides a comparable substitute, and because it requires only mapped information, may be more widely applicable.

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subjected to the Agency's peer and administative review and approved for publication.

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Influence of Egg Characteristics on Survival, Growth and Feeding in Larval Walleye (*Stizostedion vitreum*)

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A subsample of eggs from 109 walleye (*Stizostedion vitreum*) from Crean Lake, Saskatchewan, was sieved soon after fertilization to yield four groups of eggs ranging in size from 4.86 to 5.62 mm^3. Overall egg size tended to correspond to yolk and oil volumes. At hatching, larvae from the eggs with the least yolk and oil were significantly shorter than larvae from all other egg groups. At the end of the experiment, 13 d after hatching, the larvae from the eggs with the most yolk were significantly larger than larvae from the other egg groups. Differences in the mean amount of food ingested by larvae were related to initial egg size. The yolk of the smallest eggs was deficient in the (*n*-3) series of polyunsaturated fatty acids. Larvae from these small eggs had a high level of major body deformities and a mortality rate which reached 100% by day 10. Small egg size in itself did not appear disadvantageous; larvae from a different stock (Lake of the Prairies, Manitoba) which produced eggs with a mean size less than that of the smallest sieved eggs, had survival and growth rates comparable with those of the larger Crean Lake eggs.

Un sous-échantillon d'oeufs de 109 dorés bleus (*Stizostedion vitreum*) du lac Crean, Saskatchewan, a été tamisé peu de temps après l'insémination; quatre groupes ayant entre 4,86 à 5,62 mm^3 ont été obtenus. Le volume global des oeufs tendait à correspondre au volume du vitellus et des huiles. A l'éclosion, les larves provenant des oeufs qui avaient le moins de vitellus et d'huile étaient significativement plus petites que celles provenant des autres groupes d'oeufs. A la fin de l'expérience, 13 d après l'éclosion, les larves rovenant des oeufs qui avaient le plus de vitellus étaient significativement plus grandes que celles provenant des autres groupes d'oeufs. Les différences de quantité moyenne d'aliments ingérés par les larves étaient en fonction avec la taille initiale des oeufs. Le vitellus des oeufs les plus petits présentait des déficiences dans la série (*n*-3) des acides gras polyinsaturés. Les larves de ces petits oeufs avaient beaucoup de malformations importantes et un taux de mortalité qui avait atteint 100 % au jour 10. En soi, une petite taille pour les oeufs ne semble pas être désavantageuse : des larves provenant d'un stock différent (lac des Prairies, Manitoba) qui produisait des oeufs dont la taille moyenne était inférieure à celle des plus petits oeufs tamisés, avaient un taux de survie et de croissance comparable à ceux des larves du lac Crean qui provenaient des gros oeufs.

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Previous work has revealed there is heavy mortality of larval walleye (*Stizostedion vitreum*) in the interval between yolk consumption and complete oil globule absorption (Li and Mathias 1982). This period coincides with the onset of exogenous feeding which must begin almost immediately after the yolk is consumed as irreversible starvation (Blaxter and Hempel 1963) is probably reached quickly (J. A. Mathias, N. L. Loadman, and G. E. Moodie unpubl. data).

Food requirements of larval walleye are fairly well defined in terms of particle number and kind (Mathias and Li 1982). Despite seemingly adequate diets, many cultured larvae fail to feed while others feed but grow poorly. Our observation that many larvae appear destined to be poor feeders from the very onset of exogenous feeding led us to consider the effect of egg characteristics on subsequent growth, survival, and feeding. We wished to know whether larger eggs and more particularly, those with larger yolk and oil globule (OG) volumes produced more successful larvae than eggs with smaller energy reserves. We also compared the pattern of OG consumption of larvae which were growing well with those growing poorly.

Egg fatty acid composition is known to influence egg quality in other teleosts (reviewed by Leray et al. 1985). Fatty acid analyses were therefore performed on yolk and oil of eggs sampled approximately 72 h after fertilization.

Methods

Eggs were obtained from a subsample of the eggs from 109 females, fertilized by sperm from a similar number of males at Crean Lake, Saskatchewan on May 4, 1986. The eggs were immediately transported to the Methley Beach research station at Dauphin Lake, Manitoba where a subsample was separated

into three groups by means of an adjustable sieve. The intermediate sized eggs were discarded, leaving the small and large diameter eggs from the extremes of the egg diameter distribution. This procedure was executed twice to produce two groups of small and two groups of large eggs. Immediately after sieving, the yolk, OG, and total egg diameter of 50 eggs from each group were measured with an ocular micrometer. Diameters were converted to volumes by assuming the structures were spherical. Data from additional groups of unsorted eggs from Lake of the Prairies, Manitoba (LOP) and Swan Creek, Manitoba (SC) are included in some comparisons to provide a preliminary indication of the magnitude of variation caused by interpopulation differences relative to those caused by intrapopulation differences in egg size.

The eggs were incubated in 780-mL hatchery jars and given the routine care normally provided walleye eggs. Upon hatching (14 d after fertilization) 3000 larvae from each group (four Crean and one LOP) were counted by hand into five $60 \times 60 \times 40.5$ cm all-glass aquaria containing 126 L of filtered, aerated pond water. Water temperature was measured three times a day. Temperature ranged from 19 to 25°C ($\bar{x} = 22.3$) during the experiment. Temperature variation among aquaria was never >1°C with no consistent differences between tanks. Debris on the aquaria bottoms was siphoned off three times daily and, in addition, 27.5 L were removed and replaced with pond water each day. The larvae were fed zooplankton which passed through a 505-μm mesh sieve. Food was provided once or twice daily at densities of 156–585 animals·L^{-1} ($\bar{x} = 356$).

Survival was monitored by counting the number of dead bodies siphoned from the bottoms of the aquaria. The accuracy of the method was assessed at the end of the experiment by comparing the number of survivors alive in the aquaria with those predicted from the body counts. Growth, yolk, and OG consumption, and feeding behaviour were monitored by means of daily samples of 20 larvae from each tank. Total length, yolk, and OG length, and depth were measured to the nearest 0.01 mm with an ocular micrometer. Skeletal development was examined in subsamples of five larvae stained (Dingerkus and Uhler 1977; modified by Brown 1987) to reveal cartilaginous and calcified structures.

Total growth and yolk and OG consumption curves were compared by means of linear regression following transformation as described with the results. Stomach contents were identified, counted, and total lengths were recorded. The pattern of oil consumption and feeding behaviour in larvae which grew rapidly and those which failed to thrive were compared retrospectively by splitting the daily samples of normal larvae into a "fast growing" group of larvae longer than the median length of all larvae in the sample and a "slow growing" group of larvae that were shorter than the median. Individuals with lengths exactly equal to the median value were excluded. The OG volume and composition of the stomach contents of the slow and fast growing groups were compared in both stocks.

Possible causes of mortality were sought by comparing moribund to normal larvae. Moribund larvae were those lying on the bottom, with a heartbeat but incapable of movement or sustained swimming. Moribund larvae were removed during siphoning of the aquaria at 0800, 1600, and 2200 h. Relative growth of moribund larvae was assessed by comparing the number of moribund individuals shorter than the mean length of normal larvae to the number longer than the mean length of normal larvae sampled on the same day.

Lipids were extracted from eggs approximately 72 h after fertilization. The embryos had developed to the early epiboly stage (Fig. 7a, McElman and Balon 1979). Aliquots of fertilized egg samples (1.1–1.6 g) were homogenized in 10 mL of Buffer T (Idler et al. 1979). The homogenizer was rinsed with a further 10 mL of Buffer T and the combined homogenates were centrifuged at 30 000 × G for 1 h at 4°C. The floating oil was removed with two 5-mL washes of petroleum ether (30–60°C). Lipid extracts of the infranatant (crude yolk plus blastoderm) were prepared by the method of Bligh and Dyer (1959). Aliquots of the oil and yolk + blastoderm samples were saponified and fatty acid methyl esters prepared using BF_3-methanol (Wiegand and Idler 1985). Class analysis was performed by thin layer chromatography (TLC) and charring (Wiegand and Idler 1982). Since steryl esters and wax esters do not resolve clearly in the TLC solvent system employed (hexane:diethyl ether:acetic acid, 80:20:1), TLC was performed on the nonsaponifiable fractions to ascertain the presence of sterols and fatty alcohols. Other aliquots of fertilized egg samples were frozen at −80°C for subsequent determination of total dry weight and gravimetric determination of total egg lipid after extraction by the method of Folch et al. (1957). Oil mass was also determined gravimetrically. The eggs used for total dry weight determinations were counted to provide an estimate of total dry weight per egg.

Fatty acid methyl esters were separated on a 30 m, 0.25 mm bore capillary column coated with a 0.25-μm film of Durabond 225 (J&W Scientific), using a Varian 3300 flame ionization gas chromatography system equipped with a recording integrator. The temperature program was: 170°C for 5 min, 170–215°C at 2.5°C·min^{-1} followed by a 17-min hold at 215°C. The injector split ratio was 40:1 and the hydrogen carrier flow was 35 cm·s^{-1}. Fatty acid methyl esters were identified by comparison of retention times with authentic standards (Sigma Chemical Co., Nu Chek Prep Inc.) and by spiking with the same standards. Fatty acid methyl esters from cod liver oil (Sigma) and from defined mixtures of animal and bacterial origin (Supelco Inc.) were used as secondary standards (Ackman and Burgher 1965; Ackman et al. 1967). Further identifications were made by separation factor analyses (Jamieson 1975) and equivalent chain length calculations. These analyses were performed after isothermal chromatography on the same column at 200°C using adjusted retention times calculated relative to 18:0 (Ackman and Eaton 1978). Homogeneity of fatty acid methyl ester preparations was confirmed by TLC prior to injection into the gas chromatograph.

Results and Discussion

Sieving produced four groups of eggs with different mean total, yolk, and OG volumes and different dry weights (Table 1). The mean sizes of the two groups of small eggs were significantly different from each other as were the means for the two groups of large eggs even though the sieve settings used to separate the two groups of small and large eggs were identical. This may have resulted from having taken the first lot of eggs (L01 and S18) from the uppermost stratum of eggs in the hatchery jar while taking the second lot (L19 and S40) from near the bottom of the jar. The yolk and OG volumes of the S18 eggs were significantly (t-test, $p < 0.001$) lower than those of all other groups. The yolk volume of the L19 eggs was significantly (t-test, $p < 0.001$) greater than that of all other

TABLE 1. Mean volume of eggs, yolk, and OG in seived Crean Lake eggs (S18, S40, L01, L19) and length (total) of larvae on days 1 and 13 after hatching. Standard deviation in parentheses.** indicates dimensions significantly ($p < 0.001$) different from all other groups; *indicates significant ($p < 0.01$) size differences among larvae (t-tests). Sample sizes of eggs: 50 per group, Crean larvae >20 per group, LOP larvae 10 per group. Egg volumes were determined 48 h after fertilization. Lake of the Prairies (LOP) and Swan Creek (SC) eggs are shown for comparative purposes but were not included in the overall study.

	Volume (mm^3)				Larval length (mm)	
Group	Egg	Yolk	Oil globule	mg Dry weight·egg^{-1}	Initial	Final
LOP	4.79 (0.55)				7.81 (0.25)	12.04 (0.67)
S18	4.86 (0.45)	1.86 (0.17)**	0.24 (0.03)**	0.733	7.44 (0.20)*	
S40	5.06 (0.51)	2.01 (0.19)	0.26 (0.03)	0.782	7.52 (0.20)	12.02 (0.53)
L19	5.53 (0.36)	2.22 (0.20)**	0.27 (0.03)	0.842	7.70 (0.15)	12.25 (0.50)*
L01	5.62 (0.40)	2.03 (0.18)	0.26 (0.03)	0.810	7.60 (0.15)	11.99 (0.56)
SC	6.87 (0.73)	2.29 (0.29)	0.32 (0.1)			

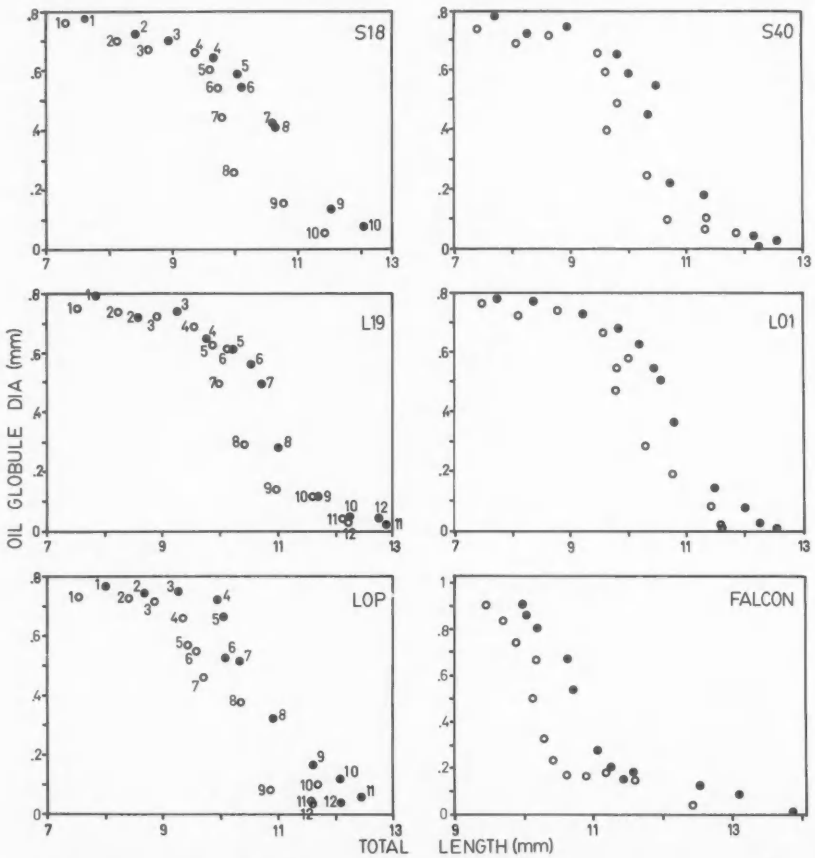

FIG. 1. Decrease in OG volume with increase in body length in fast and slow growing larvae from Crean and Lake of the Prairies (LOP). Open circles indicate slow-growing larvae, solid circles indicate fast growing larvae. Numbers beside circles indicate larval age (d).

groups. The L19 eggs also had the largest OG's, however, the difference was significant only with respect to the S18 eggs.

Variation in initial egg and yolk volume had no significant effect on the rate of yolk consumption and depletion. The rate of depletion of the OG appeared related to initial OG volume: Larvae from the smallest egg group (S18) had the fastest rate of OG consumption, however, the difference was not significant. The volume of the OG in slow growing larvae which were shorter than the median length was consistently less than that of rapidly growing larvae (Fig. 1). The slopes of the regression lines of length ($\log_e$) on OG volume of slow growing larvae were steeper than those of faster growing individuals, indicat-

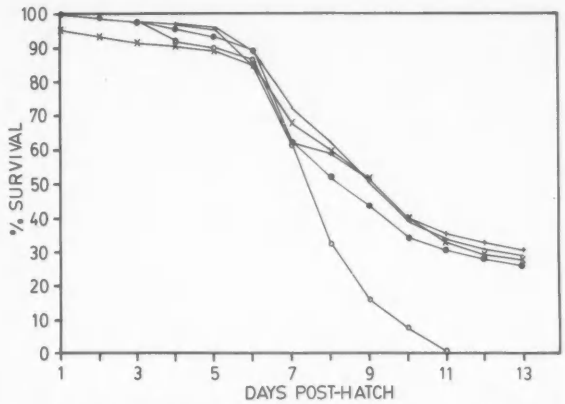

FIG. 2. Survival of Crean Lake and Lake of the Prairies (LOP) larvae from hatching until day 13. S18: (○), S40 (+), L01 (−), L19 (●), LOP (x).

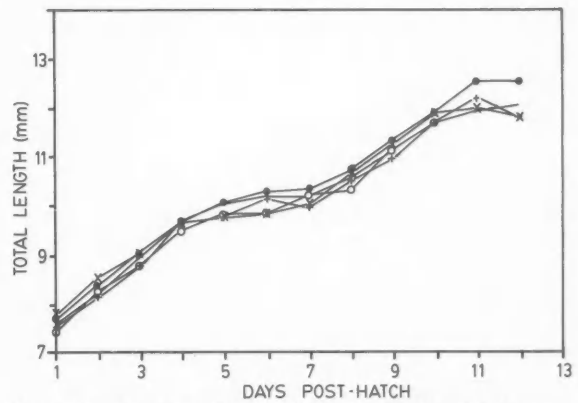

FIG. 3. Growth of Crean Lake and Lake of the Prairies (LOP) larvae from hatching until day 13. S18: (○), S40 (+), L01 (−), L19 (●), LOP (x).

ing that prior to and after the start of exogenous feeding the OG was consumed more rapidly in larvae which were growing poorly. However, the differences in the slopes were not significant. The OG represents an energy reserve which is available during the initial phase of exogenous feeding when the larva's ability to locate and capture adequate numbers of prey may be marginal. The rate of oil utilization seems related to larval well-being. In *Morone saxitalis* larvae consume the oil supply most rapidly when feeding while retaining the oil when starving (Eldridge et al. 1980). Our results show walleye larvae which are growing well delay their utilization of the oil while slower growing larvae use the oil more rapidly. The ratio of oil volume to length, especially in the intermediate period of oil utilization, can thus be taken as an indicator of larval vigor; larvae with a high ratio of oil per unit length are probably in better condition than those of the same size but with less of the OG remaining.

The pattern of survival (Fig. 2) was typical of walleye larvae. Survival was high until the transition to exogenous feeding after which various problems including failure to feed and cannibalism produced severe mortality. The survival of larvae from all egg groups was similar for the first 7 d. During the first 5 d 12–82% of the mortality was attributable to major body

TABLE 2. Rate of formation of the hypural bones and caudal fin rays among larval groups from days 6 to 8. Values indicate mean number of skeletal elements. Lines connect means which do not significantly differ (Fisher's restricted LSD, $p < 0.05$) from each other. Data from Brown (1987).

	Hypural bones				Caudal rays			
Days	S18	S40	L19	L01	S40	S18	L01	L19
6	3.0	3.2	3.6	4.0	0	0.4	1.0	1.0
7	4.6	4.2	4.8	4.4	3.4	4.4	3.8	5.4
8	5.6	6.0	6.4	6.4	6.8	8.2	8.6	8.6
Overall	4.4	4.7	4.9	4.9	3.4	4.3	4.5	5.0

deformities. Ninety-six percent of all deformities were observed during this 5-d interval. Spinal curvature and abnormalities involving the heart region, yolk, OG, and the mouth were the most common defects. These deformities were significantly (chi-square analysis, $p < 0.025$) more common (63%) among larvae from the smallest eggs (S18) (43–57% for other Crean groups). As the larvae became dependent on exogenous feeding, those derived from the eggs with the least yolk and oil (S18) suffered much heavier mortality than those from the other five groups. Similarly, only the very smallest eggs of *Leuciscus leuciscus* (Mann and Mills 1985) were significantly different from larger eggs in terms of starvation time. Small egg size appears to have negative consequences in several species. Resistance to starvation is poorer among larvae from relatively small eggs of *Etheostoma spectabile* (Marsh 1986), *Oreochromis mossambicus* (Rana 1985), *Clupea harengus* (Blaxter and Hempel 1963), and *Salmo trutta* (Bagenal 1969). The sole advantage reported for small eggs, better tolerance of low oxygen levels (Beacham and Murray 1985), may pertain primarily to species such as salmonids which produce large eggs.

The growth curves of both stocks show a short plateau around days 5 and 6, during the transition to exogenous feeding (Fig. 3). Growth rates of larvae from all four Crean egg groups were significantly (regression analysis, $p < 0.05$) greater than the growth rate of the LOP larvae. The greater length we observed among larvae from the largest (L19) eggs is paralleled in salmonids (Beacham and Murray 1985; Escaffre and Bergot 1984). The former observed the relationship only when the temperature of incubation was optimal for yolk conversion. Our data are ambiguous on this point, however, the L19 larvae reached a significantly (t-test, $p < 0.001$) larger mean length at the end of the experiment (13 d after hatching) than the L01, S40, and LOP larvae. This could result because of better and earlier ability to capture food.

Distinguishing different rates of skeletal development among the four larval groups was difficult because of the long (24 h) sampling interval, nevertheless, examination of the bones of the jaw, pectoral, and caudal skeleton from days 6 to 8 showed the dentosplenial bone, Meckel's cartilage, and the pharyngeal teeth formed more rapidly in larvae from larger eggs. Three other jaw components as well as the pectoral girdle formed at the same rate in all groups. Small sample sizes precluded statistical evaluation of these differences. The rate of formation of the hypural bones was significantly (two way ANOVA, Fisher's restricted LSD, $p < 0.05$) faster among the L01 and L19 larvae. Differences in the numbers of caudal rays were also significant but the relationship among the large and small larvae was less clear-cut (Table 2).

TABLE 3. Fatty acid composition of oil samples of Crean Lake walleye eggs[a].

Fatty acid[b]	Group S18	S40	L19	L01
14:0	2.4	2.4	2.5	2.5
16:0	7.3	7.3	7.3	7.2
16:1 (*n*-9)	2.6	2.4	2.5	2.5
16:1 (*n*-7)	11.5	11.3	11.3	11.5
18:0	0.6	0.6	0.6	0.7
18:1 (*n*-9)	17.4	17.1	17.1	17.5
18:1 (*n*-7)	2.8	2.8	2.8	2.8
18:2 (*n*-6)	6.3	6.3	6.3	6.3
18:3 (*n*-6)	0.5	0.5	0.5	0.5
18:3 (*n*-3)	4.9	4.9	4.9	4.7
18:4 (*n*-3)	2.2	2.1	2.2	2.2
20:4 (*n*-6)	2.1	2.1	2.1	2.1
20:4 (*n*-3)	1.7	1.8	1.8	1.8
20:5 (*n*-3)	4.5	4.7	4.7	4.6
22:5 (*n*-6)	1.8	1.8	1.8	1.8
22:5 (*n*-3)	2.5	2.6	2.7	2.6
22:6 (*n*-3)	21.8	22.2	21.7	22.4
Total saturates	10.7	10.7	10.9	10.8
Total monoenes	35.1	34.4	35.0	35.1
Total (*n*-6)	11.4	11.5	11.5	11.5
Total (*n*-3)	38.0	38.6	38.3	38.5
Total unknowns	4.8	4.8	4.5	4.1

[a]Compositions are weight percent. Values are means of duplicate assays.
[b]Only fatty acids comprising ≥0.5% in any one sample are listed.

TABLE 4. Fatty acid composition of crude yolk plus blastoderm samples of Crean Lake walleye eggs[a].

Fatty acid[b]	Group S18	S40	L19	L01
12:0	3.9	3.0	2.3	3.0
14:0	3.5	3.0	2.5	2.9
15:0	0.5	0.3	0.3	0.4
16:0	21.6	17.4	17.8	17.9
16:1 (*n*-9)	2.3	1.5	1.5	2.0
16:1 (*n*-7)	4.5	3.0	3.5	3.5
16:1 (*n*-5)	0.5	0.4	0.4	0.4
17:0	0.5	0.4	0.5	0.5
18:0	5.8	5.3	4.6	3.9
18:1 (*n*-9)	10.8	8.7	8.9	8.6
18:1 (*n*-7)	2.9	2.6	2.5	2.3
18:2 (*n*-6)	2.1	1.6	2.0	1.8
18:3 (*n*-3)	1.8	1.5	0.5	2.0
18:4 (*n*-3)	0.8	0.6	0.7	0.7
20:0	2.1	1.7	0.7	2.2
20:4 (*n*-6)	2.3	3.7	4.3	3.4
20:4 (*n*-3)	0.6	0.6	0.7	0.6
20:5 (*n*-3)	3.3	4.9	5.7	4.7
22:2 (*n*-6)	0.6	0.2	0.2	0.7
22:4 (*n*-6)	1.6	1.7	0.5	1.6
22:5 (*n*-6)	1.0	1.3	1.5	1.3
22:5 (*n*-3)	1.2	1.5	1.7	1.3
22:6 (*n*-3)	15.3	24.9	29.8	24.6
Total saturates	37.9	31.1	28.6	30.6
Total monoenes	21.5	16.7	17.3	17.2
Total (*n*-6)	8.2	9.0	9.1	9.3
Total (*n*-3)	23.3	34.2	39.4	34.2
Total unknowns	9.2	9.0	5.6	8.7

[a]Compositions are weight percent. Values are means of duplicate assays.
[b]Only fatty acids comprising ≥0.5% in any one sample are listed.

After the start of exogenous feeding, significantly more (chi-square goodness of fit, $p < 0.005$) moribund larvae smaller than the mean length of normal larvae were found in the S40, L01, L19, and LOP groups. The frequency of small moribund larvae in these groups ranged from 78–85% ($n \geq 72$). Moribund larvae from the S18 eggs deviated from this pattern in that approximately equal numbers (45:47) were smaller and larger than the mean length of normal larvae. Moribund and normal larvae from the smallest (S18) eggs were virtually the same size. By growing at the same rate as normal larvae, the S18 moribund larvae differed significantly (chi-square 2×5 contingency table, $p < 0.005$) from the relatively slower growing moribund larvae in the other groups.

The proportion of larvae with full stomachs indicates larval well-being. The food density provided resulted in 90.4% of larvae having full stomachs. Larvae with empty stomachs were evenly distributed between the slow- and fast-growing components of all groups (Crean and LOP). Problems arise as the proportion of larvae with full stomachs declines. Vigorous fish with empty stomachs are prone to cannibalism; 27% of attacking larvae had empty stomachs while less vigorous and smaller larvae with empty stomachs become potential victims of attack; 76% of victims had empty stomachs and were significantly (chi-square 2×2 contingency table, $p < 0.01$) smaller than their attackers.

The number of food items eaten by larvae was correlated with the size of the egg from which they hatched. Two-way ANOVA (fixed effects model; days × egg size groups) to compare the number of food items in the guts of larvae from the onset of feeding (day 5) until day 10, showed a highly significant effect of egg size. The mean numbers of food items per larva averaged over days (S18 = 6.2, S40 = 6.3, L01 = 8.8, L19 = 9) was significantly greater (Fisher's restricted LSD, $p < 0.005$) between the small and large eggs. There were no differences in the taxonomic composition or sizes of the items eaten.

Digestive capability in walleye is present prior to complete absorption of the yolk (Mitchell et al. 1986) therefore the very limited exogenous feeding prior to consumption of the yolk may be a consequence of the relatively large zooplankton consumed by walleye (Li and Mathias 1982). Walleye larvae feed on organisms which are difficult to capture due to their size and speed. Among first-feeding larvae, the frequency of prey capture ranges from 17–52% of attempts, depending on experimental conditions (J. A. Mathias and N. L. Loadman, unpubl. data). Relative to other species, this is an intermediate success rate (Meyer 1986). Walleye larvae do not resort to very small food items even in the face of a shortage of their preferred size range of prey; rotifers and nauplii <150 μm were ignored even at densities of 27 000·L^{-1} (unpubl. data). The restricted range of particle sizes which can be consumed make walleye larvae still more prone to starvation and problems associated with this are well known to culturists (Krise and Meade 1986; Hokanson and Lien 1986).

Lipid comprised 36–40% of the dry weight of the four groups of Crean Lake eggs, about 67% of which was oil. The oil was approximately 55% triglyceride with the balance consisting of both steryl and wax esters. The yolk plus blastoderm was primarily polar lipid. The oil globule lipids in the four Crean Lake groups were rich in C_{16} monoenes and in (*n*-3) polyunsaturated fatty acids (PUFA) (Table 3). Compared with egg oil of rain-

bow trout fed a cod liver oil diet (Leger et al. 1981), the walleye oil had lower proportions of saturates and linoleic acid and higher concentrations of linolenic acid. The lack of major differences between the four oils indicates the failure of the S18 group to survive cannot be attributed to oil composition.

The poor performance of the Crean Lake S18 larvae and comparatively good performance of the L19 larvae were associated not only with differences in size but also with differences in the yolk plus blastoderm fatty acid profiles (Table 4). The S18 eggs had low levels of (*n*-3) PUFA in this fraction while levels in the L19 eggs were high. Low levels of (*n*-3) PUFA have been associated with impaired development, reduced hatching success, and poor larval viability in experimental studies on a number of species (reviewed by Leray et al. 1985). Our data suggest that (*n*-3) deficiency may be a problem in some eggs from wild populations as well. Since these analyses were performed on eggs selected by size from a pooled sample, it is not known whether the S18 eggs were derived from a small number of females or if most females produce some poor eggs. These findings invite further study of the problem.

The superior survival of the LOP larvae from eggs smaller than the S18 eggs also suggests that the problems with the latter lay in their size or composition relative to the norm for Crean Lake rather than their small size itself. This emphasizes the need to determine the cause of variation in egg size, that is, whether some small eggs are a component of the spawn of all females, the product of smaller females only, or of females whose ova are immature. Evidence in the literature for a variety of species supports and contradicts all of these possibilities.

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Can We Estimate the True Weight of Zooplankton Samples after Chemical Preservation?

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Zooplankton are collected and sorted into two size fractions from which samples are randomly alloted to a battery of commonly used preservation techniques. We determine dry weight, ash content, and caloric content. We compute organic and inorganic losses of the samples to examine potential causes of variation in dry weight estimates. Treatments are: no preservation, preservation with one of three chemicals (75% ethanol, 5% or 10% buffered formaldehyde), preservation for 1 or 66 wk, and oven- or freeze-drying. Overall dry weight losses are independent of preservation methods. Chemical preservation reduces dry weight by 37 to 43%. Organic and inorganic losses range from 25 to 33% and 73 to 82%, respectively. Because inorganic losses are large, chemical preservation increases the caloric content of samples by 13 – 27%. Dry weight losses are somewhat size-dependent (37 versus 43% for the large and small size fraction respectively, after 66 wk of preservation). A regression of percent dry weight losses on body length (in millimetres) is obtained for our data, and published reports where formaldehyde is used as a preservative. It is: $\ln[\text{dry weight loss}] = 4.149 - 0.576\ \text{length}^{0.333}$. This relationship can be used to adjust the weight of zooplankton samples which have been preserved chemically. A survey of studies published in 1983 indicates that most authors did not adjust for dry weight losses due to preservation.

Nous soumettons un échantillon de zooplancton préalablement réparti en deux classes de taille à un ensemble de méthodes de préservation fréquemment utilisées. Nous examinons l'effet de ces méthodes sur les composantes organiques et inorganiques des échantillons (viz. à partir d'une détermination des poids secs et des contenus en cendres et en calories) afin de déterminer les causes potentielles de variation des estimés de poids secs du zooplancton. Les méthodes utilisées sont: pas de préservation, préservation dans l'éthanol (75%) ou la formaldéhyde (5 ou 10%), préservation pour une durée de 1 ou de 66 sem, et séchage au four ou lyophilisation. Dans l'ensemble, les pertes en poids sec ne dépendent pas des méthodes de préservation. La préservation chimique réduit les poids secs de 37 à 43%. Les pertes en matière organique et inorganique sont de l'ordre de 25–33% et 73–82%, respectivement. Comme les pertes en matière inorganique sont considérables, le contenu calorifique des échantillons augmente de 13 à 27%. Les pertes sont moins considérables dans le cas de la plus grande classe de taille (37.3%). Une relation entre les pertes en poids sec (en pourcentage) et la taille des organismes (en millimetres) est établie à partir de nos résultats et d'autres résultats où on a utilisé la formaldéhyde comme préservatif: $\ln[\text{perte en poids sec}] = 4.149 - 0.576\ \text{Longueur}^{0.333}$. Nous proposons que le poids original d'un échantillon de zooplancton soit déterminé en corrigeant le poids sec de l'échantillon préservé à l'aide de cette relation empirique. Une revue d'études publiées en 1983 indique que l'on ignore généralement les pertes de poids sec dues à la préservation.

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Weight is a measure of the size of an animal which is often used to assess production, or to quantify energy glow in aquatic communities. In most studies, it is not possible to weigh or dry zooplankton specimens immediately after collection, so they are preserved for later weight determination. However, the presence of oil on the surface of chemically preserved zooplankton samples suggests leaching of organic matter and a concomittant decrease in dry weight. Such losses have been confirmed in several studies (Morris 1972; Durbin and Durbin 1978; Omori 1978; Salonen and Sarvala 1980; Williams and Robins 1982).

To avoid this bias, Steedman (1976) recommends that the weight of preserved zooplankton be determined by reference to the relationship between the length and the weight of fresh animals. However this method requires that: (1) the length of each specimen be measured accurately, (2) the relationship be established for each species or type of organism, (3) shrinkage be estimated for corrective purposes, and (4) the relationship be reassessed frequently because the physiological condition of animals changes seasonally. This "length analysis" method which is used commonly in biological oceanography is extremely time consuming.

Other approaches have been proposed. The first one consists in improving preservation methods at a primary level, by finding better preservation methods, i.e. methods which minimize dry weight losses of preserved samples. Freezing was considered as an alternative, but reported losses are high (58%; Williams and Robins 1982). Jones (1976); Salonen and Sarvala (1985); and Kimmerer and McKinnon (1986) have tried a combination of freezing and glutaraldehyde fixation. Unfortunately, the amount of dry weight lost through this method has not been established clearly; carbon losses are negligible (Salonen and

TABLE 1. Summary of published estimates on dry weight losses due to formaldehyde preservation in zooplankton.

Organisms	Formaldehyde (%)	Duration (d)	Dry weight loss (%)	Length (mm)
Mysid				
Neomysis integis[1a]	20	21–35	9.6	17.0
Euphausiid				
Nematoscelis difficiles[2]	20^{b+*}	105	12.2	18.5
Freshwater Cladocera				
Ceriodaphnia lacustris[3]	6^{+}	0–105	47.0	0.5
Daphnia parvula[4]	4^{s+}	310	45.5	0.5
Daphnia parvula[4]	4^{s}	310	23.2	1.3
Marine copepods				
Acartia clausi[5]	6^{b*}	227–340	29.5	1.1
Acartia clausi[6]	weak	?	37.0	0.5
Pontella mediterranea[7]	5^{b+}	2–31	22.5	3.7
Calanus helgolandicus[8]	4^{b*}	153	63.8	1.7
Calanus helgolandicus[8]	4^{b*}	153	38.4	2.3
Calanus helgolandicus[8]	4^{b*}	153	18.6	2.9
Calanus helgolandicus[8]	4^{b*}	153	23.5	3.3
Calanus helgolandicus[8]	4^{b*}	153	23.5	3.3
Calanus cristatus[9]	10^{h*}	30	30.9	8.1
Calanus cristatus[9]	10^{h*}	30	34.1	8.8
	10^{h*}	30	18.3	8.2
Eurytemora affinis[10]	4^{h+}	549	30.1	1.2
	4^{h+}	549	35.0	0.7
Calanus sinicus[11]	4^{bh*}	0–180	31.0	3.1
Chaetognath				
Sagitta nagae[11]	4^{bh*}	0–180	23.0	17.2
Fish				
Clupea harengus[12]	8^{b*}	10	20.3	9.7
Clupea harengus[12]	8^{b*}	10	9.0	11.3

[a] = Neomysis is benthonic, * = with seawater, + = with freshwater, [s] = with sucrose, [b] = with borax, [h] = with hexamine.

[1]Fudge 1968, [2]Hopkins 1968, [3]Schram et al. 1981, [4]Pace and Orcutt 1981, [5]Durbin and Durbin 1978, [6]Landry 1978, [7]Champalbert and Kerambrun 1979, [8]Williams and Robins 1982, [9]Omori 1970, [10]Böttger and Schnack 1986, [11]Omori 1978, [12]Hay 1984.

Sarvala 1985; Kimmerer and McKinnon 1986), but nitrogen losses can be large (Kimmerer and McKinnon 1986). Furthermore, Salonen and Sarvala (1985) report that some organisms are not preserved at all by this method. Consequently it is not likely that this more expensive chemical will supplant formaldehyde as a general fixative in biology (Steedman 1976). The second approach consists in experimentally measuring dry weight loss due to chemical preservation by reference to the wet weight of fresh specimens or samples, and to use this information to estimate the original weight by applying an appropriate correction factor to weight after preservation. This "weight analysis" method does not appear reliable however, because published estimates of dry weight loss due to chemical preservation range widely (from 9.0 to 63.8%; Table 1). The variation could be due to differences in the biochemical composition of the organisms (species, body size, physiological condition) and/or methodological differences (length of preservation, concentration, or type of chemical preservative, buffering agent, weighing, drying, or rinsing method. Because these factors are confounded within and across studies, it is not possible to draw firm conclusions.

In this paper, we sort natural zooplankton into two size fractions and determine wet weight, dry weight, ash content, and caloric content of samples randomly assigned to a battery of commonly used treatments: no preservation, preservation in 75% ethanol or 5 or 10% buffered formaldehyde, preservation for 1 or for 66 wk, and oven- or freeze-drying. The size fractions used cover the size composition of the food of marine larval fishes (microzooplankton). In this way, we hope to understand how preservation techniques affect the organic and inorganic components of zooplankton samples, and to correct for dry weight losses due to preservation when computing the energy budget of larval fishes.

Materials

Zooplankton were collected at the mouth of the Shippegan Channel, Baie des Chaleurs (47°51′20″N; 64°44′00″W), between 14:00 and 15:00 on July 19, 1984. We towed a plankton net (with an 80-μm mesh and a 30-cm opening diameter) at low speed, near the surface; animals collected in eight successive tows were put into large (40-L) thermostatic containers and immediately brought back to the laboratory.

The sample was sorted into two size fractions with the help of sieves (250 and 500-μm mesh). The small size fraction was composed primarily of naupliar stages of copepods (*Oithona* sp., 32.7%; *Temora longicornis* Müller, 24.5%; *Pseudocalanus minutus* Krøyer, 6.1%) and of small copepodites (*Oithona* sp., 36.7%). On average animals measured 0.21 mm (SD = 0.05, n = 34, range = 0.13–0.32; cephalothorax lengths). *Ceratium* sp. was abundant. The large size fraction was composed primarily of copepodites (*Oithona* sp., 26.8%;

TABLE 2. Mean values ± SD (sample size) for percent dry weight (mg dry weight·mg^{-1}(%)), ash content (mg ashes·mg dry weight^{-1}(%)), and caloric content (calories·mg dry weight^{-1}) of the small size fraction (individuals about 0.2 mm in length) of a zooplankton sample before or after chemical preservation.

	Not preserved	Preserved
Dry weight	6.1 ± 0.66(4)	3.5 ± 0.26(8)
Ash content	36.4 ± 5.3(3)	16.9 ± 4.6(6)
Caloric content	3332 ± 282.9(7)	4561 ± 403.6(12)

Temora longicornis Müller, 19.1%; *Pseudocalanus minutus* Krøyer, 16.7%; *Eurytemora herdmani* Thompson and Scott, 15.7%; and rarely *Acartia* sp., *Calanus* sp. or a harpacticoid copepod, 1.4% each) and some naupliar (14.3%). On average animals measured 0.49 mm (SD = 0.16, n = 66, range = 0.18–0.72). *Ceratium* was also present.

Methods

All materials were sorted, rinsed with fresh water for about 10 s, and left to drain on the sieves for 15 min. Samples were scooped up with a spoon and put in weighing trays. The trays were chosen at random (by rolling dice) and their contents assigned to be or not be preserved, to be preserved with one of three types of chemical preservatives, to be preserved for 1 or for 66 wk, and to be freeze- or oven-dried prior to weight determination. Wet weight was determined by weighing the samples in random order. The weight ranged from 2.9 to 4.5 and from 3.4 to 7.9 mg for the small and large size fraction, respectively. Because of the small size of the organisms, water trapped between individuals can result in overestimating the wet weight of the samples. This bias would occur regardless of preservation method however, so it is only significant when comparing results among animals of different sizes, or when comparing studies where wet weight is measured on individual organisms to other studies where wet weight is measured on groups of organisms.

Samples not destined to be preserved were put to dry immediately. Samples to be preserved were put into jars containing appropriate amounts of 75% ethanol, or 5 or 10% sea water formaldehyde buffered with borax. At the end of the preservation period, samples were filtered on Millipore filters with a vacuum pump. Each sample was rinsed with 50 mL of distilled water (to wash out the salt). The materials were pushed off the filter into a weighing dish with a spatula. Residues remaining on the filter were washed off with distilled water into the dish. Samples were then put to dry. For oven drying, the temperature was always 60°C and drying was for 48 h or until a sample reached a stable weight.

To estimate ash and caloric content of samples, we compacted dried zooplankton samples into pellets for use in combustion experiments. Pellets were dried in the oven and weighed. Ash contents were determined by burning pellets in a muffle furnace at 500°C, for 16 h. Caloric contents were determined by burning pellets inside a Parr 1241 adiabatic semimicro bomb calorimeter with a 1710 controller using standard methods (Atkinson and Wacasey 1976). All weighing of dry samples was carried out on a Cahn electro-balance (model 27). Ash was weighed to the nearest 0.1 μg.

Dry weight was expressed as a percent of fresh weight. Weight losses were obtained by comparing percent dry weight of preserved samples to that of unpreserved samples. Ash content or inorganic content was expressed as a percent of total dry weight and the organic content was taken to represent the remaining percent of the dry weight of the sample. Caloric content was expressed in calories per total dry weight of the sample. Analysis of variance was used throughout for comparison. Because our organisms were collected on a single occasion and that samples were randomly allocated to a battery of treatments, treatments are many and replicates few. We therefore pooled samples when the probability of a difference was ≥0.20 to increase statistical power (Myers 1972).

Results

To test for effects of drying method on estimates of percent dry weight, ash content and caloric content, we only used the samples that had been preserved for 1 wk. The samples to be freeze-dried after 66 wk of preservation were lost accidentally. Drying method did not significantly affect estimates of percent dry weight, ash content, and caloric content (paired t-test; $p > 0.25$ for the three cases). We then pooled all data with respect to drying method and analyzed results by size fraction.

Small Size Fraction (individuals≈ 0.2 mm in length)

Preservation significantly affected percent dry weight, ash content, and caloric content of samples after 1 wk ($p < 0.01$). There was no significant difference due to type of chemical preservation ($p > 0.25$) or to length of preservation ($p > 0.72$, 0.21, and 0.43 for percent dry weight, ash content, and caloric content, respectively). All nonsignificant results were pooled as shown in Table 2. Percent dry weight is 42.6% lower in preserved samples than it is in unpreserved samples. Organic and inorganic losses are estimated to be 25 and 73.4%, respectively. Because losses in the inorganic component is much higher than those in the organic component, we expect the number of calories per gram of dry weight to increase in a sample. The magnitude of this increase can be predicted. If we assume that organic materials of high or low caloric value leach out of a sample in the same proportion, the caloric content will increase according to the following ratio: (proportion of the organic component of the sample after preservation) ÷ (proportion of the organic component of the sample before preservation). For the small size fraction, we predict a 30.7% increase in caloric content. The value observed is 36.9% (from Table 2). Schindler et al. (1971) reported a 16% increase in the caloric content of the freshwater copepod *Diaptomus oregonensis* after formaldehyde preservation, and Atkinson and Wacasey (1983) reported increases ranging from 2.26 to 12.15% for benthic marine invertebrates after formaldehyde and/or alcohol preservation.

Large Size Fraction (individuals≈ 0.5 mm in length)

Preservation significantly affected percent dry weight, ash content, and caloric content of the samples after 1 wk ($p < 0.01$). Although there was no significant difference in percent dry weight or ash content due to type of chemical preservative ($p > 0.48$ and 0.42, respectively), caloric content differed significantly with type of preservation ($p < 0.01$). The percent dry weight of samples preserved for 1 wk varied significantly from that of samples preserved for 66 wk ($p < 0.05$). Ash content did not vary significantly with respect to length of preservation ($p > 0.20$) whereas caloric content did ($p < 0.01$). Those results are reported in Table 3. Percent dry weight is

TABLE 3. Mean values ± SD (sample size) for percent dry weight (mg dry weight·mg^{-1}(%)), ash content (mg ashes·mg dry weight^{-1}(%)), and caloric content (calories·mg dry weight^{-1}), of the small size fraction (individuals about 0.5 mm in length) of a zooplankton sample before or after chemical preservation. Results were pooled when $p > 0.20$.

	Fresh sample	Time (wk)	Preserved samples: 95% ethanol*	5% formaldehyde	10% formaldehyde
Dry weight	11.89 ± 1.05(4)	1	*	*	*
		66	Δ	Δ	Δ
Ash content	19.5 ± 0.65(3)	1	+	+	+
		66	+	+	+
Caloric content	4517 ± 61(9)	1	5464 ± 165(4)	5523 ± 48(4)	5740 ± 88(5)
		66	5141 ± 41(2)	5628 ± (1)	5631 ± (1)

* = 7.46 ± 0.16(4), Δ = 6.75 ± 0.36(3), + = 6.10 ± 1.39(7).

43.2 and 37.3% lower in preserved samples than it is in unpreserved samples after 1 and 66 wk of preservation, respectively.

After 1 wk of preservation, we estimate that the organic and inorganic losses are 26.6 and 81.5%, respectively. After 66 wk of preservation, the comparative figures are 33.4 and 80.5%. Based on these figures, we expect caloric contents to increase by about 17% after chemical preservation (calculations and assumptions as in the previous section). We observe 21.5% (pooled results for 1 and 66 wk of preservation; from Table 3). The systematic underestimation of the increase in the caloric content of preserved zooplankton samples based on percent dry weight and ash content estimates suggests that organic substances of low caloric content leach out significantly more from samples than substances of high caloric content.

A statistically significant difference in the caloric content among different types of preservatives is problematic: it was not corroborated by a corresponding difference in percent dry weight ($p > 0.25$). Since dry weight appears lower in the sample preserved in alcohol than in the samples preserved in formaldehyde after 66 wk of preservation, and since others have reported that specimens preserved in ethanol lose significantly more weight than those preserved in formaldehyde (mysid: Fudge 1968; tubicifid: Howmiller 1972; chironomids: Dermott and Paterson 1974), we suspect that dry weight losses are larger when preserving large zooplankton in ethanol instead of formaldehyde.

Discussion

We sorted a single zooplankton sample into two size fractions and determined fresh weight, dry weight, ash content, and caloric content of samples randomly assigned to a battery of commonly used treatments: no preservation, preservation in 75% ethanol, 5% or 10% buffered formaldehyde, preservation for 1 or for 66 wk, and oven- or freeze-drying. Our objective was to understand the effects of some usual chemical preservatives and techniques on the organic and inorganic components of zooplankton samples, so that organismic and methodological factors could be sorted out and dry weight losses adjusted for.

Findings and Evaluation of the "Weight Analysis" Method

We observe 37 to 43% dry weight losses due to chemical preservation. Overall these losses are relatively independent of methodological biases (type of chemical preservative, length of preservation, or drying method). The following precautions could help standardize results: (1) preserve all samples in buffered formaldehyde solutions. Ethanol is not recommended because it may lead to greater dry weight losses in large organisms. Buffering is preferable because the pH of formaldehyde varies considerably among brands or stocks (Markle 1984). Hexamethylene-tetramine should be preferred to borax because it reduces dry weight estimates less significantly (Omori 1978). (2) Preserve large specimens for longer periods of time. A standard preservation period of 6 mo should ensure that the weight of all zooplankton specimens reach a stable value. Data in Steedman (1976) and Schram et al. (1981) indicate that most weight losses occur in the first 30 d of preservation, and a minimum preservation period of 1 mo would be suitable for small organisms. (3) Use oven- or freeze-drying. If drying in an oven, temperature should not be too high (say 60°C) or drying period too long (Lovegrove 1966).

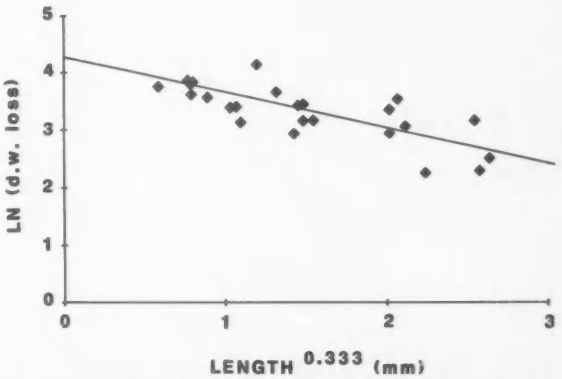

FIG. 1. Natural logarithm of dry weight loss (%) due to formaldehyde preservation as a function of the cubic root of body length (mm) for zooplankton (this study and Table 1). The regression line is statistically significant.

It is noteworthy that dry weight losses are 37 and 43% for the large and the small size fraction, respectively after 66 wk of preservation. This size dependency is observed in spite of the narrow size range used in the experiments. Combining our data with those in Table 1, we can show an overall dependency of dry weight losses on body length (in millimetres) for all results dealing with formaldehyde preservation: ln(dry weight loss) = 4.149 − 0.576 Length$^{0.333}$; (equation 1 and Fig. 1; $p << 0.01$; DF = 1,22; $r^2 = 0.58$; length range: 0.2–18.5 mm). This equation could be used to correct the weight of preserved

zooplankton samples although a substantial amount of variation remains unexplained (42%). A better fit might be obtained if we use another metric for body size (e.g. body width, body weight, total organic content, total inorganic content). For example, if the organic composition of zooplankton increases with increasing body size, and if organic losses are proportionately smaller than inorganic losses as in our experiments (25–33 versus 73–82%), an inverse relationship between dry weight losses and body size could be expected. Unfortunately, for the reports summarized in Table 1, ash content and body size do not follow this simple pattern. Another possibility is that leaching is a diffusive process which is size- and time-dependent (e.g. diffusive properties depend on distance and on chemical structures which change during the fixation process). In that case, dry weight losses due to preservation could depend on the smallest dimension of the organism (rather than its linear dimension) and on some other characteristics of the body plan (e.g. whether the largely inorganic skeleton is external or internal).

Even if the fit of the relationship is improved by using another metric however, a substantial amount of variation will remain unexplained because species specific, season specific, and/or sex specific effects appear to be important. For example, the relationship between dry weight losses and body size obtained intra-specifically is steeper than that shown in Fig. 1 in all cases (*Daphnia parvula*, Pace and Orcutt 1981; *Calanus helgolandicus* Williams and Robins 1982; *Clupea harengus*, Hay 1984; *Eurytemora affinis*, Böttger and Schnack 1986). We also observe that adult copepods yield greater dry weight losses than immature individuals of similar size (Omori 1970, Williams and Robins 1982; Table 1); this may be due to the loss of lipids which adults store in substantial amounts (Atkinson and Wacasey 1983). In that case, the trend described by equation 1 would be the result of observations derived from a series of L-shaped intra-specific relationships; a substantial amount of variance must therefore remain unaccounted for. The predictive power of equation 1 is thus limited and it should be used with caution. Yet we feel that a restricted application of equation 1 is warranted because it is a substantial improvement on current practices.

To estimate how better estimates of dry weight loss might impact on literature reports involving zooplankton, we searched the 1983 biological abstracts under the key words dry weight, length-weight, and biomass (Falk-Petersen et al. 1982; Kane 1982; Tande 1982; Caminas 1983; Confer et al. 1983; Garnås 1983; Haberman 1983; Hamilton and Powles 1983; Hisashi and Shiroh 1983; Huang 1983; Joh and Uno 1983; Johnson 1983; Lindahl and Hernroth 1983; Maranda and Lacroix 1983; Okamoto and Horie 1983; Pedrós-alió and Brock 1983; Percy and Fife 1983a,b; Rankin et al. 1983a,b; Tremblay and Roff 1983; Vass and Zutshi 1983; Worobec 1984). Of the 22 references culled, 11 used chemically preserved specimens. Of these 11 references, only three corrected for losses due to preservation methods and these added 33% to the sample weight. For the kinds and sizes of organisms considered, our data indicate that a 33% correction still underestimates dry weight losses due to preservation.

We therefore recommend the following procedure when estimating dry weight of preserved zooplankton samples. (1) Sort zooplankton specimens by size. (2) Apply the "length analysis" method for large organisms which are relatively rare, and yet represent a large proportion of sample weight. (3) Apply the "weight analysis" method for small specimens, correcting for dry weight losses with equation 1. This, for example, yields multiplicative factors of 1.42 and 1.24 for mesozooplankton (size $\leq$1.0 mm) and macrozooplankton (1.0$<$ size $\leq$10.0 mm), respectively. One may also have to adjust dry weight estimates upwards by 33% to allow for dry weight losses due to rinsing in marine samples (Omori 1978), although some of the losses due to rinsing may have been taken care of by the above correction. More research is needed in this area.

We believe that this simple procedure will help provide more precise estimates of the dry weight of preserved samples of zooplankton than is currently achieved. For studies with large numbers of small organisms where time constraints preclude application of a complete dry weight loss correction procedure, it is imperative that researchers adequately document methodologies so that later investigators can apply post hoc corrections.

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PERSPECTIVES

Current Approaches to Multispecies Analyses of Marine Fisheries[1]

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There is an increasing tendency to recognize that the dynamics of a fishery are not solely determined by intrinsic population factors, but that the external environment, which can include the effects of other species in the biotic community, plays an important role in determining the yield performances of marine fish stocks. Recognition of the role of external factors represents an important departure from the single-stock concepts of fishing theory, which have traditionally been used to manage marine stocks. We review a variety of examples of recent initiatives in the application of community concepts in marine fisheries, setting these in the context of four arbitrary categories which range from statistical description through multispecies extensions of single-stock fishing theory, to integral applications of community theory. Taken together, there is substantial evidence that the application of multispecies concepts to marine fisheries is now a major focus of international effort. The multispecies approach offers identifiable promise in resolving some outstanding problems, but it is not clear yet what the major consequences will be, in terms of changes in the ways that fisheries will be managed in the future.

On pense de plus en plus que la dynamique d'une pêcherie n'est pas déterminée uniquement par des facteurs démographiques intrinsèques, mais que l'environnement extérieur, notamment les effets d'autres espèces sur la communauté considérée, a une influence importante sur le rendement des stocks de poissons de mer. La reconnaissance de l'influence des facteurs externes constitue une démarcation nette par rapport aux notions de stock unique qui ont été régulièrement utilisées pour la gestion des stocks de mer. Nous passons en revue différentes tentatives d'appliquer des concepts de communautés aux pêcheries en mer, que nous présentons selon quatre catégories fixées arbitrairement, c'est-à-dire une description statistique, l'application à plusieurs espèces de la théorie d'exploitation de stocks uniques ou encore des applications intégrales de la théorie des communautés. Quand ces exemples sont pris ensemble, on voit bien que l'application de notions multispécifiques aux pêcheries suscite beaucoup d'intérêt sur le plan international. Cette approche multispécifique ouvre visiblement la voie à la résolution de problèmes, mais on ne voit pas encore les grandes conséquences qu'elle aura dans la façon de gérer les pêches.

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Fishing theory, the conventional management approach taken to most of the commercially important stocks of marine fishes in the north-temperate zone, is essentially a set of single-stock methods. Allowable yield is assumed to be an equilibrium function of the stock itself, and the effects of external factors, including community effects, are not explicitly incorporated in the calculation systems. This approach has apparently worked well in some instances, for example Daan (1987) and Dickie et al. (1987) note that analyses based on multispecies considerations seem broadly congruent with the results of more traditional techniques, but the recent scientific literature indicates a growing concern that fishing theory is deficient because it neglects ecological considerations that are fundamental to the process of fish production. Steele and Henderson (1984) have gone so far as to suggest that fishing theory was developed at a time when the major fisheries were relatively close to equilibrium, thereby fortuitously approximating some of the major assumptions of the approach.

This apparent weakness of an accepted management procedure is by no means an emerging awareness of a new problem; multispecies considerations in resource exploitation have a long historical record, which we do not consider here. In its modern form, concern about the effects of community interactions in fisheries dates back at least to Larkin (1963) and Riffenburgh (1969), and much has since been published in a similar vein, including consideration of the effects of abiotic environmental

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factors on fisheries yield. Nevertheless, perhaps because the exciting developments of fishing theory diverted our full attention for a time, it is only in recent years that the ecological basis of fish production processes has begun to attract general interest among marine fisheries scientists, marking the onset of a period of revolutionary change in thinking. For many years, the concepts that guided the development of the scientific basis for management of marine fisheries remained curiously separate from the mainstream of ecological thought, to a much greater extent than was the case with freshwater fisheries science. Perhaps the genesis of freshwater fisheries science was closely enough tied to the limnological milieu that the effects of environmental factors were not so easily forgotten; certainly it is true that the limited economic worth of individual freshwater fisheries did not encourage general use of the data-intensive and expensive management procedures that could be accepted for the more valuable marine stocks.

An interesting counterpoint to this theme has been the recent attention to appropriate techniques for appraising the species-rich fisheries of tropical marine systems. The age-dependent, stock-intensive procedures used for temperate fisheries are clearly not appropriate in these circumstances, leading to initial emphasis on aggregative Schaefer-type models, followed more recently by expanding ingenuity in the construction of length-dependent procedures designed to obviate the substantial processing costs of the age-dependent techniques traditionally used for north-temperate fisheries (Pauly and Morgan 1988).

As always, it is easy to make the obvious criticisms of established procedures, such as traditional fishing theory, but it is much harder to offer constructive alternatives. Despite the recent revolution espousing ecological thinking in fisheries ecology, the fact is that new procedures for managing fisheries, founded in ecological process as opposed to the population considerations of fishing theory, are as yet quite tentative and undeveloped, although promising. Consequently, the following survey should be regarded as a progress report, not a final product.

Our purpose is to summarize the progress that has been made to date in seeking broader, ecologically-founded supplements to fishing theory in the marine context, and to suggest where these various ideas seem to be leading. The literature on ecosystem effects in marine fisheries is already voluminous, as it is in related ecological disciplines. Accordingly, this is a necessarily selective, but we hope comprehensive overview of conceptual developments. In the next section, we provide examples of a wide variety of approaches that have been taken to the analysis of multispecies fisheries, in order to provide a concrete basis for subsequent discussion.

General Approaches to Date

We begin with four arbitrary but convenient categories for describing the main approaches that have been taken to multispecies considerations in marine fisheries, overlooking some of the obvious intergradations between any two of our categories. The descriptive detail we provide under each category reflects our perception of the amount of explanation necessary, and has no bearing on its putative importance. Rather than select conventional terms (e.g. empirical, mechanistic, etc.) for our choice of categories, with the attendant risk of seeming to prejudge the conclusions, we chose to use terms that are as neutral as we could devise. Our views on the most promising approaches have been set out elsewhere (e.g. Ryder et al. 1981; Kerr 1982; Dickie and Kerr 1982), and will not be repeated here. With these qualifications, we suggest the various approaches that have been taken with respect to the community aspects of marine fish production can be usefully summarized as follows.

1. Descriptive Multivariate

A rich variety of powerful statistical and other techniques exists for the purpose of discerning and describing pattern in ecological (and other) data, and these have been widely applied to marine fisheries statistics, usually obtained from commercial catch reports or research vessel surveys (e.g. Tyler 1971; Gabriel and Murawski 1985; Mahon 1985). Significant clusters of species can usually be discerned with such techniques, but these often seem to owe more to the effects of physical environmental factors than to obvious biological interactions (Mahon 1985; McKelvie 1985). Comparable approaches can be taken to the analysis of time-series data (e.g. Saila and Erzini 1987). The value of these techniques is not so much in their ability to discern pattern in "noisy" data, but in their capacity to identify putative functional groupings amenable to subsequent causal analysis, as exemplified in category 3 below.

In a more analytical vein, Sissenwine et al. (1982), using simple Gaussian models, were unable to discern evidence of significant species interaction in their empirical analysis of the marine fishery statistics of the northeastern U.S.A., although they carefully qualified their expectations because of the limitations imposed by the available data and the analytical procedures used. Although this example could as easily have been included in our next category, it is described here because of its heavy empirical emphasis and simple dynamical content. Within the stated limitations, their analysis suggests that it is inappropriate to search for evidence of community interaction in terms of a simple, pairwise coupling of the stocks comprising a given fishery. Interestingly, however, they do provide qualitative evidence that strong year–classes of piscivorous species did not coincide, suggesting that species interactions could indeed be at work in organizing the structure of this major fishery complex.

Given the obvious limitations of commercial catch statistics, or survey data, over the enormous spatial scales associated with the major marine fisheries, it is perhaps unreasonable to expect very much light to be shed on the existence and intensity of community interactions solely on the basis of descriptive empirical procedures. Usually, however, the patterns discerned in descriptive analyses are seen as a useful preliminary to process-related studies, rather than as ends in themselves, leading to the next category in our classification scheme.

2. Dynamical Multivariate

There is little question that recurrent patterns exist in the organization of at least some of the stocks comprising marine fish communities. The real questions are whether, and how, these regularities can be turned to the advantage of fisheries managers. Much attention has been given to this matter in recent years, as various investigators have sought to link functional relationships among species stocks with the observed patterns in the commercial landings. In general, the analyses we regard as belonging to the present category build upon knowledge of single-species processes to deduce the behaviours of multispecies systems (c.f. Shepherd 1988). As such, the analyses tend to depend less on community-level observations, and more on extensive observations at the level of the individual stocks.

The multinational attack on the North Sea fishery is a useful example for consideration here. On the basis of their pioneering simulation studies of fisheries ecosystems, Anderson and Ursin (1977) were apparently the first to suggest a scientific basis for managing this major fishery complex as an ecosystem. The challenge was taken up, primarily in the form of age-structured techniques known as multispecies virtual population analysis and multispecies cohort analysis, whch is not unlike running the Anderson and Ursin simulation model in reverse, utilising the accrued catch statistics to structure the calculation systems (Ursin 1982). The essence of translating the age-structured population techniques to a multispecies context entails a detailed knowledge of who eats whom, enabling the natural mortality matrix to be partioned to include a predation component (e.g. Gislason and Helgason 1985). To this end, a stomach analysis program was mounted that Daan (1986) describes as a breakthrough, in terms of its potential impact upon fisheries management knowledge of the North Sea production system. Ursin (1982) provides a useful discussion of the tradeoffs between data requirements and ease of analysis in making a selection among the preceding model types.

The partitioning of natural mortality to include an observable predation component, even if restricted to the fish community itself, is an obvious improvement over existing single-species techniques. At present, it is not clear whether, or to what extent, this important initiative will influence fisheries management in the North Sea because it is still in the developmental stages for that system (Daan 1987). Nevertheless, preliminary results (e.g. Gislason and Helgason 1985) suggest that predation causes very much higher and potentially more variable mortality rates on the prerecruits than single-species analyses allow for, with commensurate effects on the ensuing recruitment and exploitation patterns in the fishery. If this result is confirmed, and the bioenergetics analysis of the Georges Bank, Gulf of Maine fishery (Sissenwine 1984, 1986) independently points to the same conclusion, then it may well be that inclusion of species interactions (exclusively piscivory by the fishes themselves in the present examples) are an essential addition to conventional management procedures.

3. Multivariate System

The examples we offer in this category depend upon aggregations of data derived from sources such as conventional catch statistics. Perhaps the ultimate expression of the approach is the multispecies Schaefer model (FAO 1978), in which the total fish biomass of all species is treated as if it were a single stock. This approach often works surprisingly well, although not necessarily for reasons that are well-understood (FAO 1978). The examples we offer here are chosen at an intermediate level of aggregation (i.e. at a level somewhere between that of the individual stock and the total catch of all species of fishes).

The possible approaches for aggregation of fisheries statistics are in principle limited only by the ingenuity of the analysts, but in practice by the need for some acceptable measure of objectivity. The study by Ralston and Polovina (1982) of the carnivorous, demersal species taken by the deep-sea, hand-line fishery in Hawaii is exemplary, both for its use of objective clustering techniques and its careful attention to statistical aggregation theory. Their results indicate that the 13 species which figure in the fishery are reliably separable, on the basis of depth distribution, into three groups which are stable in space (i.e. among separate fishing grounds), and with time. The conclusion reached, that aggregation at the intermediate level of cluster groups tends to explain more of the variance than either single-species or multi-species Schaefer analysis, indeed suggests the existence of community-level effects in the determination of observed fishery yield, although alternative possibilities cannot be definitely ruled out.

The preceding example dealt with a production system that was apparently stable in time and space, within the limitations of the available data. Our next case-history deals with the vexing question of species replacement, especially among the marine pelagic fishes, which exhibit an apparent sequence of alternating abundances, with characteristic periodicities in the order of 40 or 50 yr (Steele and Henderson 1984). Daan (1980) looked at this question on a pair-wise basis, and found the evidence for equivalent species replacement to be sometimes present, if somewhat equivocal. Silvert and Crawford (1989) used a somewhat more aggregated approach, plotting the dominant pelagic against the combined catch of the other pelagic species in the fishery. Their results, for five major pelagic assemblages, suggest a limit-cycle pattern of alternating abundances. Interestingly, their attempts to simulate these observed behaviours required both the use of lagged response of fishing effort to changes in abundance, as well as the incorporation of biological interactions (apparent competition for common prey resources) among stocks. Neither factor, operating alone, could suffice in itself to account for the observed fluctuations of abundance.

4. Integral Systems

At the larger aggregation scale of community or ecosystem behaviour, there is little question that important system conservatisms are prevalent in marine fisheries, offering support for the view that community interactions are important factors organizing the structures of fish production systems (Dickie and Kerr 1982). Total yield from a marine fish community, for example, is often relatively conservative despite high variability of the constituent species (e.g. Sutcliffe et al. 1977), just as has been observed with freshwater fisheries (e.g. Regier and Hartman 1973).

A further example of a community conservatism is the observation (Sheldon et al. 1972) that the average particle-size spectrum of offshore pelagic communities tends to be smooth and orderly. That is, the distribution of biomass concentration is a relatively flat, but slightly decreasing function of organism body size, for reasons which include trophic interactions (Kerr 1974). This suggests that biomass estimates for one segment of the biomass distribution (phytoplankton, for example), can be used to estimate the biomass of a size interval that is more difficult to estimate directly, such as fish. Fisheries estimates using this technique have been made by Ryder and Henderson (1975); Sheldon et al. (1977); and Moloney and Field (1985) showing that surprisingly good correspondance with alternative estimators can be reached on the basis of relatively little information.

The biomass spectrum in the sea spans many orders of magnitude of organism size, from bacteria through to whales. Whether the spectrum remains as orderly and well-behaved over the relatively smaller span of sizes that includes just the fishes has not been so well-studied. Pope and Knights (1982) examined the distribution of numbers at length for groundfish survey data taken over three successive years in the northern North Sea, and found remarkably uniform patterns, with the expected negative slope. Similar data, for 1 yr only, obtained from the

Faroe Bank showed a similar distribution, but with a shallower slope, indicating greater numbers of large fish and lesser numbers of small fish than in the North Sea distributions. The authors suggested that the different slopes reflected the different mesh sizes used in the two fisheries. Clearly, there is evidence that orderly size-spectra obtain over the narrower size-window comprising the demersal fishes.

It is difficult to accurately transform distributions expressed as logarithms of numbers versus body length into the conventional format of the biomass spectrum (biomass concentration versus logarithm of body size, indexed as equivalent spherical diameter), although such a transformation is at least logically possible. Accordingly, while we prefer not to attempt direct comparisons of the groundfish distributions with the conventional biomass spectrum, we can safely conclude that orderly distributions do exist within the relatively narrow size window that encompasses the trawlable groundfish. Using normalized biomass spectra, Sprules and Munawar (1986) have reached similar conclusions, that comparably characteristic biomass spectra obtain over the size-interval of the limnoplankton, for a number of diverse ecosystems, a conservatism they associate with the trophic status of the observed system. Nevertheless, although the evidence accumulates, much careful work remains to be done before the biomass-size spectrum can be routinely used as a fisheries forecasting procedure.

In particular, there are some interactions with benthic communities that need to be understood before the size-spectrum method can be used in shallow water systems, where benthic–pelagic interactions can apparently be important. The size structures of benthic communities are conditioned by the nature of the substrate, which in turn affect the communities occupying the overlying water column, resulting in the appearance of complementary size modes in the benthic and pelagic systems where shallow depths permit strong interaction (Schwinghamer 1985). In aggregate, accordingly, present evidence suggests that the characteristically uniform biomass spectrum prevails when benthic and pelagic spectra are treated together. The premise is that the biomass size spectrum promises to afford a robust approach to making predictions about fish production systems, even if some outstanding questions remain to be investigated.

Another dimension of the use of community conservatisms in assessing marine fish production emerges from the coupling of biotic–abiotic dependencies. Models of this kind most frequently take the form of empirical relations describing fish yield as functions of one or more abiotic variables, chosen a priori for their known effects on aquatic production. In freshwater lakes and reservoirs, community production is often predictable on the basis of one or more controlling or limiting abiotic factors (e.g. Ryder et al. 1974; Jenkins 1982). At the most fundamental level of organization these factors, or their surrogates, are ultimately expressions of energy and/or nutrient availability. Implicitly, the concept of biotic–abiotic dependencies may be extended to marine systems in a general sense, albeit with certain qualifications, as the latter are often more open-ended and less discrete than freshwater systems (e.g. Forbes 1887). In this respect, marine systems more closely resemble terrestrial systems than they do lakes. Nonetheless, despite the obvious differences among various types of marine and freshwater ecosystems, the distinctions are a matter of degree rather than qualitative in nature. Accordingly, it seems that previously developed models for freshwater systems depicting biotic–abiotic dependencies might be profitably applied to marine systems (e.g. coral reefs, Marten and Polovina 1982), providing that the appropriate modifications are made.

One model of this type, often applied to both lakes and reservoirs (and most recently, rivers) on a global basis, is the morphoedaphic index (MEI). This index consists of a nutrient value in its numerator, usually expressed in terms of total dissolved solids, conductivity, or phosphate concentration, and an energetic (morphometric) variable in the denominator (Ryder et al. 1974). The whole expression is sometimes scaled by a dimensionally-correct coefficient to correct for latitudinal differences related to annual heat input (e.g. Schlesinger and Regier 1982). Potential fish yield can then be estimated for any particular lake or reservoir using the MEI equation, assuming there are no limnological anomalies of inordinate weight, no unduly large cultural interventions on the abiotic system, and a harvesting pressure sufficient to stimulate density-dependent, compensatory responses among the various components of the fish community, invoking the inherent system conservatisms (i.e. an approximate constancy of total annual yield over time).

Extension of the MEI concept to marine systems is not inappropriate, and a pioneering foray was attempted for both the Zuiderzee (now a freshwater lake, the Ijsselmeer), and an estuarine portion of the Baltic (Ryder et al. 1974). In the future, more rigorous applications might, for example, advantageously utilize nitrate concentration in the numerator of the MEI expression, and mixing depth or some other index of mixing potential (e.g. Simpson and Hunter 1974) in the denominator. Climatic effects would perhaps be better expressed in terms of the mediating effects of the oceanic current systems (e.g. Collier 1970), as opposed to the latitudinal thermal coefficient applied to comparable analyses of freshwater fisheries (Schlesinger and Regier 1982). With this approach, rapid, first approximation yield estimates could be made for the various continental shelf and bathypelagic fisheries of the world oceans.

Various models of this general type have considered production of marine biota other than fishes (e.g. Hargrave 1973; Turner 1977), and many others of comparable form have been applied to fisheries production systems, but with mixed success (e.g. Skreslet 1986). Of numerous potential candidates only one, to our knowledge, is actively used as a component in formulating the management regime for a major fishery, the model of abiotic effects on capelin *Mallotus villosus* year–class formation in Newfoundland waters (Leggett et al. 1984), and this is a single-species system. It is difficult to know in what measure the dearth of applications in marine fisheries management results from lack of utility, as opposed to reluctance to accept change on the part of a necessarily conservative management structure. In this regard, it is interesting to contrast the ready acceptance of integral, empirical procedures in freshwater fisheries management worldwide, as opposed to the disposition to prefer the methods of fishing theory in marine fisheries management.

Discussion

Despite some inevitable measure of overlap, four categories of multispecies considerations in marine fisheries analysis have been identified and discussed above. All have their strengths and their weaknesses, depending upon their abilities to address the particular question at hand. From first to last, there is a general tendency for decreasing data requirements with progression through the categories, which must be balanced against the overall precision and reality of the ensuing results. Gener-

ality, on the other hand, as well as speed and ease of application, tend to increase substantially with the increasing degree of aggregation of system components in the analysis.

There are inescapable situations when scientific management must address the status of individual stocks, and there are times when the well-being of larger assemblages, the gadoids as an identifiable entity in a given region for example, should feature in management considerations. Choosing a suitable analytical technique, however, is not a simple function of the degree of ecological aggregation appropriate to the problem; it is often a complex amalgam of the degrees of freedom in the system under scrutiny, combined with the availability of useful data and the understanding necessary for its appropriate application. Given these considerations, the choice of appropriate analytical technique is not just a simple matter of choosing from an array of models which offer increasing degrees of subsystem aggregation.

In fact, the degree of subsystem aggregation in itself does not bear a close or necessary relation to the development of a satisfactory analytical solution. It is important to note that significant convergences can be discerned among the different approaches offered; it is not a coincidence, for example, that the size-dependent (integral systems) approach to estimation of early mortality (Dickie et al. 1987) has led to similar forms of mortality schedule as those obtained from the more direct, and costly, observational (dynamical multivariate) approach tried in the North Sea. Given that the integral behaviours of multispecies fisheries embody much of the information contained in the behaviours of the individual species stocks, there should be no surprise in realizing that integral methods can potentially converge on results of the same form as those obtained from single-species considerations. Moreover, it seems that such integral results can be reached more cheaply, and more quickly, than is possible with the traditional single-stock techniques of fishing theory.

Clearly, the multispecies approach to marine fisheries is now receiving considerable attention, although the field has not developed to nearly the same degree as in freshwater. In large measure, this is because the major marine fisheries are considerably more difficult to work with. They are much larger, in terms of both physical scale and ecological complexity, and are considerably more 'open' systems, in the sense that both the fishes themselves and the physical processes driving the systems are capable of extensive spatial excursions (Kerr and Ryder 1988). Nevertheless, there is little doubt that coherent multispecies behaviours are observable in marine fisheries, just as they are in freshwater.

It remains to determine the extent to which multispecies regularities result from community interactions, as opposed to other factors. Because species are caught together by the same gear does not necessarily imply strong community interactions; they may be present together, for example, merely because of some habitat requirement they share in common. Bycatch in such instances does mean that the exclusive use of single-species models is an inappropriate approach to managing the aggregate fishery, but it does not necessarily mean that the appropriate model must contain species interaction terms. Nevertheless, the evidence for strong effects of predation within major fisheries seems persuasive (e.g. Sissenwine 1984, 1986; Daan 1987), although support for strong competitive effects is somewhat more diffuse.

The concept of "competition" is of course a contentious issue in community ecology in general, being difficult to demonstrate conclusively, perhaps because organisms go to some considerable lengths to avoid experiencing its effects. Fraser (1978), to our knowledge, is one of the few to provide unequivocal evidence of the existence of competitive interaction in a fish community. It is no surprise to us that evidence of strong competitive interactions should be relatively hard to obtain. Our view is that species which have shared habitats and similar prey resources, for a very long time, would have co-evolved complementary patterns of behaviour that ensure continued coexistence, providing the basis for development of a harmonic community structure, such as is seen in freshwater systems (Ryder and Kerr 1984). At the community level, the evidence supports such a view. The variance of the total catch of all species is commonly much less that would be expected on the basis of the variability of the individual stocks, suggesting that internal interactions are at work. For another example, the uniformity of the average biomass spectrum suggests that internal interactions account for the tendency to spread biomass evenly across the possible range of organism sizes. That is, species interactions, reflecting secular changes in environmental factors, tend to fully occupy the production potential offered by a given system.

At the intermediate level of aggregation, there is also good evidence to support the view that species interactions influence the fish production process in marine systems. As noted above, species pairs sometimes exhibit replacement patterns, leading Skud (1982) to invoke the related freshwater community concepts of interactive segregation (Nilsson 1967) and dominance–surbordinance (Svardson 1976) to account for alternating abundances in some major marine fisheries. Often, however, it appears that evidence of interaction is more readily found at somewhat higher levels of system aggregation, as in the examples provided earlier. If there is any generality to be gained from the brief overview offered in the preceding section, perhaps this is it; the highest levels of aggregation are most likely to capture the salient behaviours of marine production systems. Given the substantially greater faunal diversity of marine systems, relative to freshwater fisheries, the requisite shifts in species abundance in response to environmental change are more readily available to cope with the opportunities for biological production. Where greater species richness prevails, it is reasonable to suppose that greater levels of community aggregation would be needed to provide clear evidence of interactive patterns.

Fishery exploitation is a well-recognized force in structuring or disaggregating fish communities, one which is necessarily recognized in all management models. But this introduces another very large and complex set of community concepts — concepts appropriate to the behaviour of the fishery itself. In this sense, economic considerations such as markets, prices, fuel costs, as well as some of the less tangible considerations that determine fisherman behaviour (Allen and McGlade 1986) are necessarily part of the management picture. It is true these are not ecological community concepts in the usual sense, but there is a case to be made for some strong similarities in economic and ecological concepts (Rapport 1986), so we are not straying so far from the present theme as it may seem. Nevertheless, while we flag economic considerations as germane to the multispecies context, we do so only in passing; such matters require full consideration within their own context.

If we accept the view that fisheries management is in a period of rapid change, as community concepts become incorporated with traditional approaches, it is relevant to enquire, or at least speculate, on the possible effects this may have upon the ways

that fisheries data are acquired, and analysed. With the exception of the biomass spectrum, it should be noted that most multispecies analyses of marine fisheries have tended to utilize conventional types of fisheries data, presumably for the very good reason that they are available. It seems reasonable to suggest that further progress may depend greatly on the routine acquisition of different types of data, on seasonal energy contents of different stocks and individual sizes of fishes, for example, and certainly on their trophic interactions by season and by size–class. Discards are generally poorly recorded. Much more information is also required on the other major components of marine systems, in particular how the fishes interact with seabirds, mammals, predatory invertebrates, and the largely unsampled fishes such as some of the sharks. Marine faunas are generally quite complex, compared with freshwater communities, and the commercially exploited fish stocks are only a part of this assemblage, even if we consider just the highest trophic levels. If progress is to be made in distinguishing between the effects of physical and biotic factors in organizing fisheries, many new data are required to allow bioenergetic analyses of marine fisheries. This is a well-developed field in freshwater, but quite primitive in marine systems; even the basic metabolic parameters are unknown for most of the major marine species, especially at the low temperatures seasonally experienced at temperate latitudes.

Just as there is potential for data requirements to change with the development of community models, so are changes likely in the way these models will be used to manage our fisheries. The entire area of multispecies models for management of marine fisheries is so new, and developing so rapidly, that any suggestions we offer should be regarded as merely speculative. In fact, the advent of multispecies management approaches to marine fisheries seems to have begun rather quietly, with the initiation in 1972 of the two-tiered approach to the ICNAF implementation of total allowable catch levels. The scientific basis for this approach is described elsewhere (e.g. Brown et al. 1976). By this reasoning (e.g. Byrne 1986) quotas were established for the individual species considered separately, and a second (lesser and safer) quota level for the summed catch of individual species was then imposed, thereby allowing for the effects of species interactions. From this humble beginning, much more seems possible.

Tyler et al. (1982) for example, offer the notion of assemblage production units as the focal units for management appraisal, suggesting that coherent groups of species in a given spatial zone can be effectively managed as distinct entities. If this is a viable approach, there are many additional factors that must be taken into account in making the transition to a truly multispecies view of marine fisheries. At the moment, it is much too soon to say what is likely to prove useful; but there is no question that fisheries management is entering into a new, multispecies era, in which community concepts will have a large role.

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ANNOUNCEMENTS/AVIS

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We are pleased to announce the first international symposium on "The Rainbow Trout" which will be sponsored by the international journal "Aquaculture". The Rainbow Trout Symposium will be dedicated to the discussion of the biology, production, and management of this important cultured species, particularly in the context of fish farming, but also with regard to natural resource management. The international scientific committee will be chaired by Dr. G. A. E. Gall, editor of "Aquaculture". The format of the symposium will consist of plenary sessions of invited papers and poster sessions of contributed papers. Topics covered during six plenary sessions will be:

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— economics and marketing,
— genetics and breeding,
— husbandry and management,
— nutrition and feeding,
— physiology and endocrinology.

Contributions of poster papers are encouraged for all aspects of Rainbow Trout biology, management, and production, including both wild, domestic, and managed forms of the species. The proceedings of the meeting will be published as a special volume of "Aquaculture", and reprinted in hardbound form. If you wish to receive further information about The Rainbow Trout Symposium please contact: Mr. Keith Foley, The Rainbow Trout Symposium, P.O. Box 521, 1000 AN Amsterdam, The Netherlands. Tel: (020) 586–2828, Telex: 10704, Fax: (020) 586–2580.

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Nous sommes heureux d'annoncer la tenue du premier symposium international sur "la truite arc-en-ciel" qui sera parrainé par la revue internationale "Aquaculture". Lors de ce symposium, on discutera de la biologie, de la production et de la gestion de cette importante espèce d'élevage, surtout dans le contexte de l'exploitation piscicole, mais aussi pour ce qui a trait à la gestion des ressources naturelles. Le comité scientifique international sera présidé par le Dr. G. A. E. Gall, rédacteur de la revue "Aquaculture". Le symposium sera constitué de séances plénières d'exposés de conférenciers invités et de séances d'affichage de documents préparés pour l'occasion. Les sujets traités au cours des six séances plénières seront les suivants:

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— aspect économique et commercialisation,
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— nutrition et alimentation,
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